

U.S. Army.

MP (DOCUMENT SECTION)

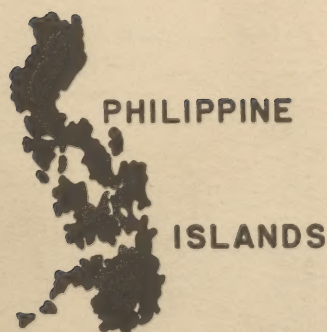
406 MEDICAL GENERAL LABORATORY

ANNUAL HISTORICAL REPORT

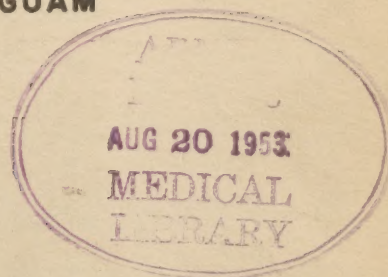
1952



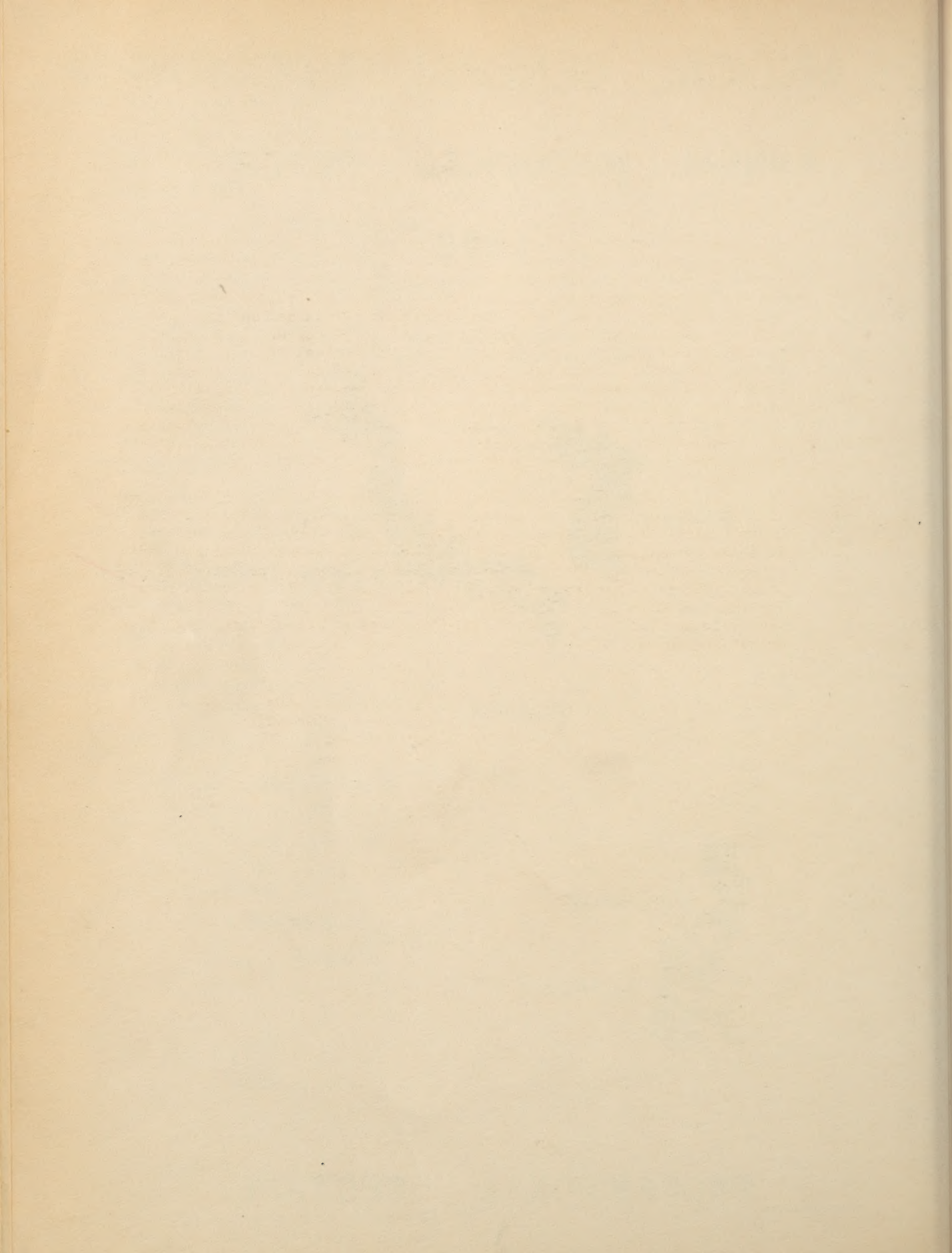
IWO JIMA



SAIPAN
TINIAN, GUAM



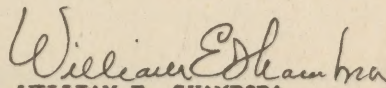
PROFESSIONAL SECTION



FOREWORD

Adequate laboratory support is essential to the early recognition, proper evaluation, and development of therapy of many medical problems whether involving a single patient or an entire community. Research and the accurate recording of observations are essential to continued medical progress. These facts have long been recognized and apply to military medicine as forcefully as to civilian medicine. It therefore becomes necessary for the military to provide medical research facilities, strategically located, to attack those medical problems peculiar to military experience because of warfare or geographical limitations of certain diseases. It is also mandatory that adequate medical laboratory facilities be available to support the clinical care of military personnel. When clinical laboratory support and research facilities can be combined an obvious economy of personnel is effected.

The following report consists of the combined statistical data and preliminary conclusions formulated by the regular staff members of the 406th Medical General Laboratory and the Far East Medical Research Unit in their normal duties during 1952. Since many research teams and individuals from the Army Medical Service Graduate School, the Office of the Surgeon General, and various medical schools in the United States have worked as members of the Far East Medical Research Unit during their visits to the Far East Command, their findings and conclusions have been summarized and are included.



WILLIAM E. SHAMBORA
Major General MC
Chief Surgeon

PREFACE

While the routine diagnostic service of the laboratory has continued at a rather even level, research activities, both in Korea and Japan, have been greatly increased during 1952. An ever increasing sense of urgency has prevailed that at least a few of the important military medical problems must be solved. This, along with fluctuations in military activity in Korea, has produced wide variation in the work load of the laboratory.

As in past years, this report is an anonymous collection of material which the editors believe to be representative of the work of the entire organization. Many persons have contributed to this joint effort which has continuously been aimed at greater knowledge of disease and injury, and better service to the doctors of the Far East Command.

The great assistance rendered this unit by other military and civilian agencies, and by many individuals, both civilian and military, not directly associated with the organization is deeply appreciated. Without this assistance the accomplishments would have been considerably more difficult and in some instances impossible.

R.P.M.

CONTENTS

DEPARTMENT OF EPIDEMIOLOGY

Hemorrhagic Fever	1
Influenza	4
Japanese B Encephalitis	8
Infectious Hepatitis	12
Streptococcal Infections	15

DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES

Routine Examinations	17
Japanese B Encephalitis - Natural History	22
Japanese B Encephalitis - Experimental Infection In Birds	28
Japanese B Encephalitis - Experimental Infection In Mosquitoes	35
Hemagglutination Inhibition Test Applied To The Serodiagnosis of JBE	36
Japanese B Encephalitis - Antibody Response To Field Vaccination	42
Normal Inhibitor In Human Serum In The Serodiagnosis Of Influenza	44
A Hepatitis Virus Of Mice	46
Ornithological Studies	51

DEPARTMENT OF ENTOMOLOGY

Statistical Summary Of Activities	57
Surveys For Ectoparasites Of Small Animals	59
Black Fly Studies	71
Studies On Biting Midges	73
Taxonomic Studies	73
Biology Of Culex Tritaeniorhynchus	76
Epidemiology Of Japanese B Encephalitis	79
JBE Experimental Infections In Mosquitoes	82
Mammals And Arthropods For Laboratory Experimentation	85
Preparation Of Biological Material	87

DEPARTMENT OF CHEMISTRY

Clinical Chemistry	89
Sodium And Potassium In Urine And Saliva	91

Urinary 11-Oxycorticosteroids	92
Serum Or Plasma Amylase	95
Toxicology	96
Method For Urine	98
Method For Blood	99
Food Chemistry	99
Allergy Investigations	103
Water Analysis	108
Miscellaneous Analyses	109
Hemorrhagic Fever	113
Study of Bank Blood Available In Korea	115
Studies of Blood From The United States	118
Paper Chromatography Of The Opium Alkaloids	118
Chromatography Of Alkaloids Extracted From Toxicologic Specimens	126
Chromatographic Purification Of Alkaloids Extracted	128

DEPARTMENT OF BACTERIOLOGY

Routine Procedures	130
Cultural Studies On Hemophilus Ducreyi	132
Bacterial Flora Of Non-Specific Urethritis	135
Cultural Patterns And Antibiotic Sensitivity Of Staphylococci	136
Fungi In Cultures Of Dermatophytoses From Americans In Japan	137
Streptococcal Pharyngitis And The Streptococci Of Powdered Milk	139
Water And Dairy Bacteriology In Japan	140
Clostridial Studies	142
Study Of Anaerobes In Freshly Incurred War Wounds	147
Sorbic Acid An Aid In Rapid Isolation Of Clostridia	157
Clostridia - Cultural Reactions, Use Of Polymixin, Isolation Technique..	158
Tuberculosis	160
Shigella Types in Japan, Okinawa and Korea	161
Phase Variation In Shigella Flexneri 4	169
The Group Factors Of Shigella Flexneri 4	172

Salmonella Types In Japan And Korea During 1952	175
Evaluation Of Shigella Typing Serum	176
In Vitro Group A Streptococcus Variation And Streptolysin Production	177
Leptospirosis In Japan	179

DEPARTMENT OF PATHOLOGY

Routine Specimens Received	181
Battle Injuries	188
Amputated Extremities	189
Frost Bite	193
Fatal Battle Injuries	195
Fat Embolization	197
Lower Nephron Nephrosis	201
Salmonellosis	208
Infectious Hepatitis	210
Parasitic Infections	211
Relapsing Fever	213
Liaison With Other Medical Units	214
Officer Personnel	215
Recommendations	215

DEPARTMENT OF MEDICAL ZOOLOGY

Routine Stool Examinations	217
Molluscacide Studies - Laboratory Screening Tests	217
Molluscacide Studies - Field Plot Dilution Tests	219
Molluscacide Studies - Evaluation Of Field Procedures	227
The Incubation Period Of The Eggs Of Oncomelania Nosophora	229
Egg Laying Tendencies Of Oncomelania Nosophora	230
Prospectus For Control Measures Against Newly Hatched O. Nosophora	233
Immunologic Resistance Against Schistosoma Japonicum	234
Investigations On Schistosomiasis In Taiwan	235
The Penetration-Time For The Cercariae Of Schistosoma Japonicum	236
The Epidemiology Of Intestinal Protozoa In Family Groups	238

A Comparison Of The Zinc Sulfate And The Formalin-Ether Technics	246
Efficiency Of The Formalin-Ether Concentration Technic	248
Mass Treatment Of Endamoeba Histolytica Carriers	254
Skin-Tests For Amebiasis	255
Immunologic Investigations On Ascaris	255
Seasonal Studies On Ascaris Lumbricoides	257

DEPARTMENT OF SEROLOGY

Routine Tests	259
Cardiolipin Microflocculation Antigen	259
Effect Of Inactivating Sera For Cardiolipin Wassermann Tests	261
Serodiagnosis Of Paroxysmal Hemoglobinuria	261
Preparation Of Rare Antisera Against Human Erythrocyte Antigens	262
Cold-Warm Agglutinins In Pregnancy	264
Rh And ABO Blood Group Distributions In Japanese	264
D ^u Antibodies In Pregnancy	265

FAR EAST MEDICAL RESEARCH UNIT

Introduction	268
Epidemic Hemorrhagic Fever	269
Surgical Research Team	277
Renal Insufficiency Center	286
Special Investigation Of Blood Usage And Blood Storage	290
Wound Ballistics and Body Armor	290
Neuropsychiatric Research Team	290

PUBLICATIONS AND MANUSCRIPTS	291
------------------------------------	-----

BIBLIOGRAPHY	294
--------------------	-----

ROSTER OF PERSONNEL OF 406TH MGL AND FEMRU	309
--	-----

DEPARTMENT OF EPIDEMIOLOGY

The Epidemiology Department of this laboratory was organized in 1951, with one medical officer assigned full time to the pursuit of epidemiologic activities. This department has been primarily concerned with providing the laboratory and preventive medicine consultants within the theater with detailed and specific information pertinent to problems which it has investigated, and no attempts at preventive epidemiology have been made. The following report presents a brief account of some of the problems which were of interest to this department and to medical personnel in the theater during 1952.

HEMORRHAGIC FEVER: The first recognized case of hemorrhagic fever among UN personnel was a single fatal case which appeared in April, 1951, and was diagnosed in retrospect by review of autopsy material after criteria for pathologic diagnosis had been established. A review of the Russian and Japanese literature disclosed the fact that armies of both nations had experienced a disease of similar manifestations in Siberia and Manchuria in the late 1930's and that considerable investigation had been conducted.^(1, 2)

With few exceptions all cases in UN troops have contracted their disease in a rather narrow region of Korea bounded on the north by the MLR, on the east and west by the coasts and on the south by a line drawn roughly from Seoul on the west to Inje and Norumegi on the east. The greatest concentration of cases has been in the immediate vicinity of the MLR, particularly the western sector. It is probable that this endemic region extends into North Korea and Manchuria though the actual data on the presence of disease in this territory is unknown.

Although cases develop throughout the year, incidence of the disease is characterized by two distinct peaks, one in the spring and the other in the fall. There appears to be fair correlation between the peaks of incidence and the dry seasons of Korea, the spring-summer outbreak declining with the onset of the heavy rains of midsummer and the fall peak declining with the advent of the snows of winter.

The etiology of the disease is not definitely known. Available evidence indicates that the agent is probably of viral or rickettsial nature and that the disease is transmitted by certain arthropod vectors. It is also probable that a reservoir of infection exists and field rodents indigenous to the endemic area are suspected of harboring the agent. The disease is almost entirely confined to the rural population. The majority of cases develop in men living in tents and bunkers in intimate contact with the ground, and a large rodent population is commonly described. Several species of mites whose monthly variation in population appears to be correlated with the seasonal peaks of incidence have been found in the endemic region of Korea. The rodents most commonly suggested as possible reservoirs of the agent are the Microtus and Apodemus agrarius.

Clinical Description - Hemorrhagic fever is a disease characterized by fever, non-specific constitutional symptoms, capillary and arteriolar damage, and impaired renal function. After an incubation period which may vary in duration from two to five weeks, the onset of symptoms occurs suddenly and with few prodromata. Illness is commonly heralded by chills, fever, malaise, retro-orbital headache, abdominal and back pain, and frequently nausea and vomiting. Weakness, syncope, and fainting, particularly when standing, are not uncommon. Blurring of vision and diplopia are sometimes seen. This initial, or febrile phase, lasts from three to eight days and is usually concluded by a rapid lysis of the fever. Toward the end of this period, conjunctival injection and edema, and petechiae of the soft palate and pressure areas of the skin frequently develop. Albuminuria invariably appears around the third day and within two days thereafter is usually 4/+. Absence of albuminuria precludes a diagnosis of hemorrhagic fever. The early febrile period is quite regularly associated with a deep flush of the face, neck, and upper thorax resembling to a great extent the erythema of sunburn.

Immediately following defervescence, some degree of hypotension is commonly seen. In the majority of cases this hypotension is mild and responds well to conventional measures. In about five per cent of the cases, true shock occurs and may result in death. This period of hypotension lasts from a few hours to two or three days following which the blood pressure gradually rises. During and following this "hypotensive phase", oliguria develops and the urine usually contains large numbers of red cells, sometimes to the extent of gross hematuria. This oliguria is not dependent upon hypotension, as it may develop in the absence of any significant lowering of the blood pressure.

At the conclusion of the oliguric period, diuresis begins suddenly, and urine volumes of 4 to 5 liters per day are not uncommon. The consequent rapid loss of fluid and electrolytes may result in serious problems of management. During or shortly before the onset of diuresis, hypertension is sometimes seen and arterial pressure in the neighborhood of 160-180/100-120 is not uncommon. Seldom is the elevated pressure high enough to be dangerous in itself, and during recovery it gradually returns to normal.

Convalescence is characterized by a return of appetite and ability to retain fluids, continuation of diuresis, and gradual return of renal function. Diminished urine concentrating ability and proteinuria may continue for several weeks after the patient is otherwise apparently well. Available data indicates that recovery, once effected, is complete. All cases have regained clinically normal renal function. It appears that some degree of immunity is imparted, as no patient has developed the disease a second time, though most are returned to duty in the endemic area.

Clinical laboratory data usually shows definite abnormalities. The white blood count often rises during the late febrile period and sometimes resembles a leukemoid reaction. Counts of 20,000 or more with many immature myeloid forms are not uncommon. Blood platelets are usually low and bleeding time is prolonged. The hematocrit usually rises during the hypotensive and oliguric phases and may fall rapidly following the onset of diuresis. Impaired urine concentrating ability is evident during the diuretic period and proteinuria is a constant finding. Blood urea nitrogen and creatinine levels rise with the onset of oliguria and continue for some time after diuresis is established.

Serum electrolytes are variable. An increase in plasma potassium is common and may be sufficient to produce electrocardiographic changes. Chlorides are frequently low. Hyponatremia sometimes develops during diuresis, and hypocalcemia is frequently seen. Total serum proteins show no consistent pattern. In general, the albumin fraction decreases until around the tenth day of illness. Recovery is accompanied by a gradual return of all these changes toward normal. The elevated BUN, proteinuria, and diminished concentrating ability may persist for several weeks after all symptoms have subsided.

Incidence - The distribution of cases and deaths during 1951 and 1952 is shown graphically in Figures 1 and 2. During 1951, 989 cases were reported and in 1952, 1,041 cases developed. The graphs for the two years must be considered together as the decline of the second (fall-winter) peak in 1951 extends into 1952. Dividing the graphs arbitrarily as follows into four periods, certain comparisons may be made:

- (1) 1 June 1951 through 8 August 1951 (spring-summer outbreak, 1951)
- (2) 15 August 1951 through 19 March 1952 (fall-winter, 1951 to 1952)
- (3) 26 March 1952 through 27 August 1952 (spring-summer outbreak, 1952)
- (4) 3 September through 31 December 1952 (fall-winter 1952 outbreak)

During the spring-summer outbreak of 1951, 91 cases were reported of which 15 died, or a case fatality of 16.5 per cent. It is likely that this high percentage of deaths resulted from a combination of factors, the most important of which was failure to recognize mild cases of the "new" disease. Delayed or traumatic

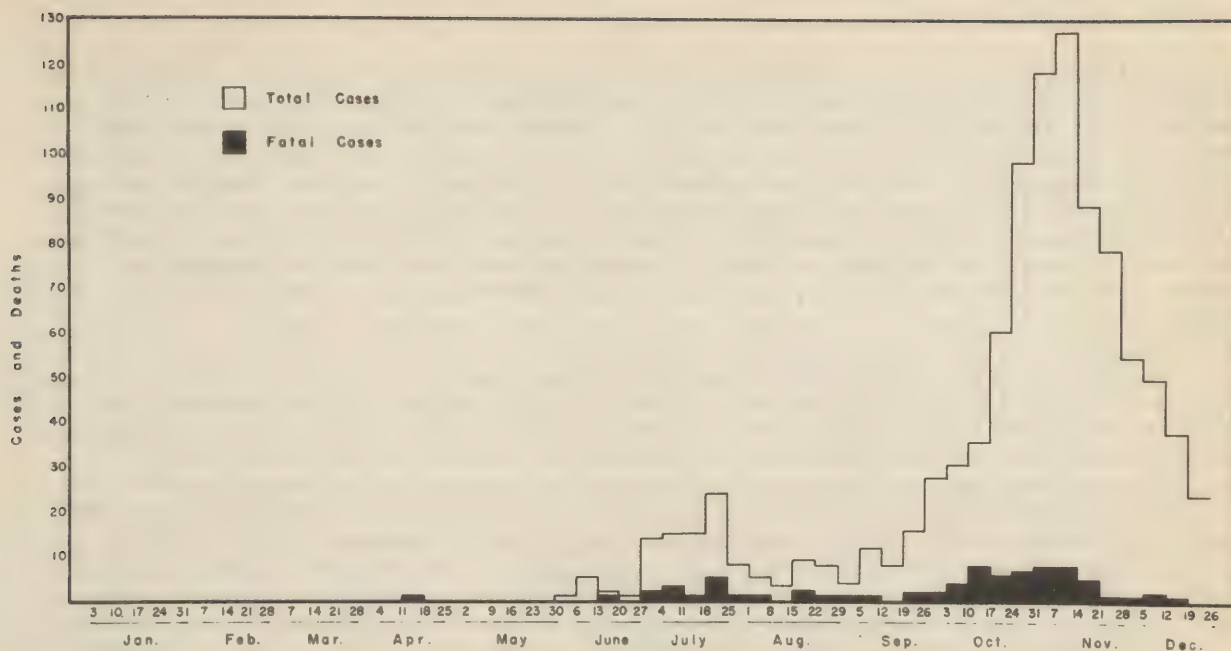


Figure 1. Incidence of Hemorrhagic Fever in Korea - All Personnel, 1951, By Week of Onset (Deaths Recorded by Week of Onset of Illness)

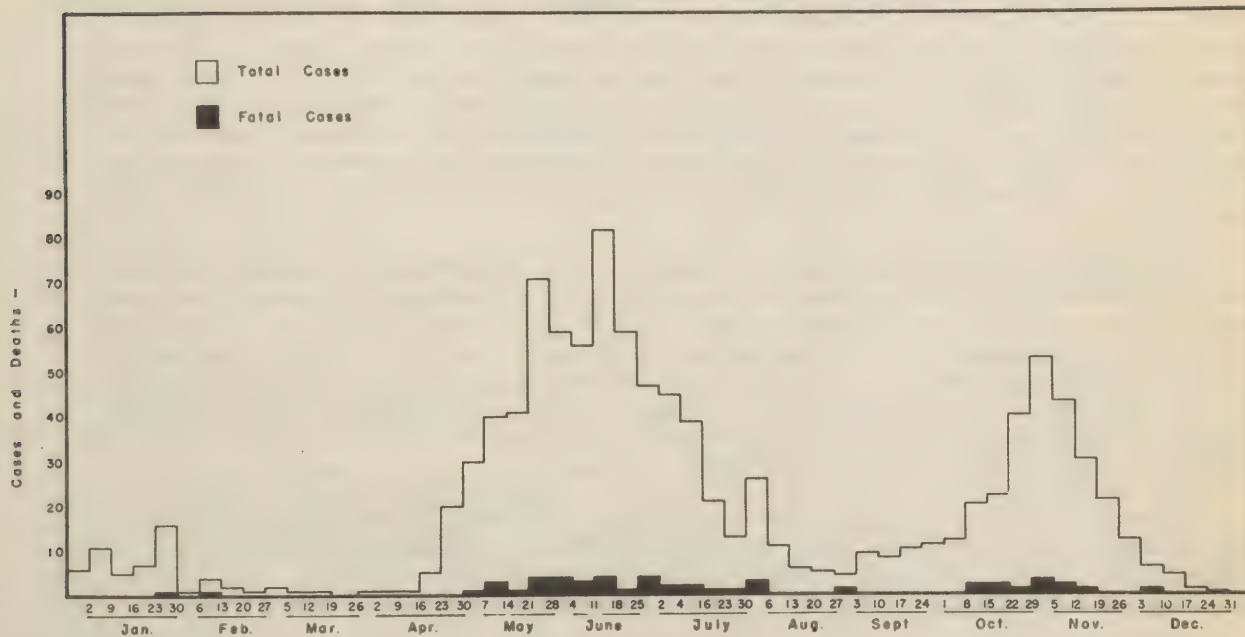


Figure 2. Incidence of Hemorrhagic Fever in Korea - All Personnel, 1952 By Week of Onset (Deaths Recorded by Week of Onset of Illness)

evacuation and improper treatment may have contributed to this high case fatality rate, though this seems less important since these conditions still prevailed in the fall-winter outbreak, when 954 cases were reported with 62 deaths, a case fatality rate of 6.5 per cent.

Dividing the graph for 1952 into two periods, a striking difference is seen. Whereas in 1951 the spring-summer epidemic was small in contrast to the fall-winter season, this situation was reversed in 1952. During the spring-summer season this year, 679 cases were reported with 34 deaths, a case fatality of 5 per cent. In the fall-winter epidemic, 306 cases were reported with 13 deaths, a case fatality rate of 4.2 per cent. The possibility of missed mild cases still exists, but familiarity with the disease makes this possibility seem unlikely. The fall in the case fatality rate observed during the two years probably reflects the improved evacuation system, earlier recognition of cases, and better understanding of the principles of proper therapy.

No single event can be ascribed as responsible for the unexpectedly small fall outbreak in 1952. It is interesting to speculate with regard to the impregnation program instituted by the Eighth Army in the summer of 1952. In a disease thought to be transmitted by arthropod vectors, attempts at control of the disease would logically include measures designed to decrease the vector and reservoir population, and to deny potentially infected vectors access to susceptible individuals. Accordingly, an active rodent control program was instituted. Impregnation of clothing with mite repellants was initiated in all units in the endemic area. Exact figures on the number of troops in the endemic area who had their clothing impregnated are unobtainable. Similarly, even among the men who subsequently developed the disease, it is difficult to arrive at accurate knowledge of the extent of clothing impregnation employed by each man. Consequently, no definite cause and effect relationship between the clothing impregnation program and the small fall outbreak can be made. However, clothing impregnation is one of the major differences between the conditions which obtained in 1951 as compared to 1952 and this could have influenced the decline in the incidence. On the other hand, no knowledge of the annual variation in incidence is available. It is possible that the pattern observed this year is a natural phenomenon, due perhaps to weather conditions and/or a small vector-reservoir population. Similarly, it is conceivable that the replacement of American troops in forward areas with ROK forces might have been a factor in the lesser incidence among Americans in 1952. Neither can a decrease in pathogenicity of the causative agent be excluded.

INFLUENZA: In October 1952, a number of cases of influenza-like disease were admitted to an air force hospital in the Philippine Islands, and between 1 October and 15 December, 178 cases were observed. These were reported as upper respiratory infections and common colds but as paired sera from a sampling of the cases began to show rising antibody titers to Type A' influenza virus, it became apparent that a high percentage of the patients actually had Type A' influenza. This fact was not recognized until the Philippine outbreak was definitely on the decline. During December, only sporadic cases were seen.

Cases of a similar nature began to appear in Okinawa in November, where again the pattern of an abrupt increase in incidence rapidly reaching a peak with a subsequent rather rapid decline in December was noted. Here, too, paired sera from a sampling of cases showed diagnostic rises in titer to influenza Type A' virus.

In early December cases appeared in Japan, first in the Tokyo-Yokohama area, but rapidly spreading to the suburbs and rural areas. The progression in Japan was irregular but generally proceeded northward.

Finally, during mid-December confirmed cases of influenza began to appear in Korea and it was feared that a major epidemic might be in progress. It is reported that large numbers of cases are now occurring in the United States and Europe and it is apparent that the disease has become pandemic.

As the epidemic in this theater is still in progress, a definitive presentation of its magnitude and distribution cannot be made at this time. However, in Figure 3, an attempt is made to show the relative time of appearance of confirmed cases of influenza in the major divisions of this command. It may be seen that the first serologically proven cases of the current outbreak developed in Okinawa and the Philippines in November. From here, the disease spread first to the Tokyo-Yokohama area of Japan, then to other regions of the islands, and finally to Korea. Actual numbers of cases serologically confirmed are also shown, arranged by location and week of onset. The data for Korea is misleading in that many suspected cases are occurring there at this time. An unknown number will subsequently be confirmed. The data accompanying paired sera submitted to the laboratory for influenza studies indicate that most of these suspected cases in Korea had the onset of their illness in late December and early January, 1953.

Figure 4, shows the relative rates of upper respiratory disease, including influenza for Japan, Korea, Okinawa, and the Philippines during 1952. It would appear from the available data that relatively few of the cases represented by the high rates in early 1952 were actually true influenza, in contrast to the current outbreak, in which a relatively large number are probably actual cases of the disease.

Clinical Course - Typically, the cases seen are characterized by prodromata resembling a common cold with the later development of severe malaise, frontal headache, and muscular soreness. Chills are not uncommon and fever up to $102 - 103^{\circ}\text{C}$ is a frequent finding. Sore throat is not prominent and there has been no increase in the incidence of beta hemolytic streptococcal pharyngitis. Cough of a non-productive nature develops on the second or third day and often persists for a week or more after the acute manifestations of illness have subsided. Occasional cases have developed bacterial pneumonia which responds well to antibiotics and no deaths from the disease among American personnel have been reported. In most instances, the disease runs a self-limited course of 4 to 6 days requiring only symptomatic care.

Serologic Typing of Paired Sera - Table 1, shows the serologic experience with influenza during 1952, as reported by the Department of Virus and Rickettsial Diseases; (See report of this department). The winter 1951-1952 season gave rise to very little influenza and the interval August through December 1951 is not represented in the Table, as during that period none of 4 paired sera received by this laboratory showed diagnostic rises in titer to influenza virus. It will be seen from Table 1, that the majority of the positive paired sera tested during the early months of 1952 showed highest hemagglutination inhibition titers to the Lee strain of virus and were, therefore, influenza Type "B" cases. During that period isolation was accomplished in two instances, both isolates being influenza Type "B" virus.

In contrast, beginning in November 1952, when the first sera of the winter 1952 outbreak began to show diagnostic rises, almost all rises were to influenza Type A. About sixty percent of the paired sera tested by the hemagglutination-inhibition method during the winter of 1952-1953 showed diagnostic rises in titer predominantly to FW1-50 (Cuppet) and, to a lesser extent, to the FM-1 strains. The remaining 40% had equal rises in titer to both FM-1 and Cuppet strains. Of 37 isolation attempts this winter, Type A virus has been isolated in six instances. Only 3 paired sera tested this season have shown diagnostic rises in titer to Type B virus. Two of these originated in Japan and one in Korea. Though the large spectrum of antibiotics currently available is capable of substantially reducing the number of deaths resulting from secondary infection, the range of virulence of the influenza virus is still not known and the disease must be considered a major potential threat. Moreover, even in the absence of any appreciable death rate the degree of morbidity associated with the infection results in a staggering loss of effective man hours in any large community where the disease reaches epidemic proportions.

For these reasons the question of immunization of the American forces in Korea arose. As it appeared that existing influenza vaccines would protect against the currently encountered strains of virus, an immunization program for the Eighth Army was initiated in January 1953. However, at the present time large numbers of suspected

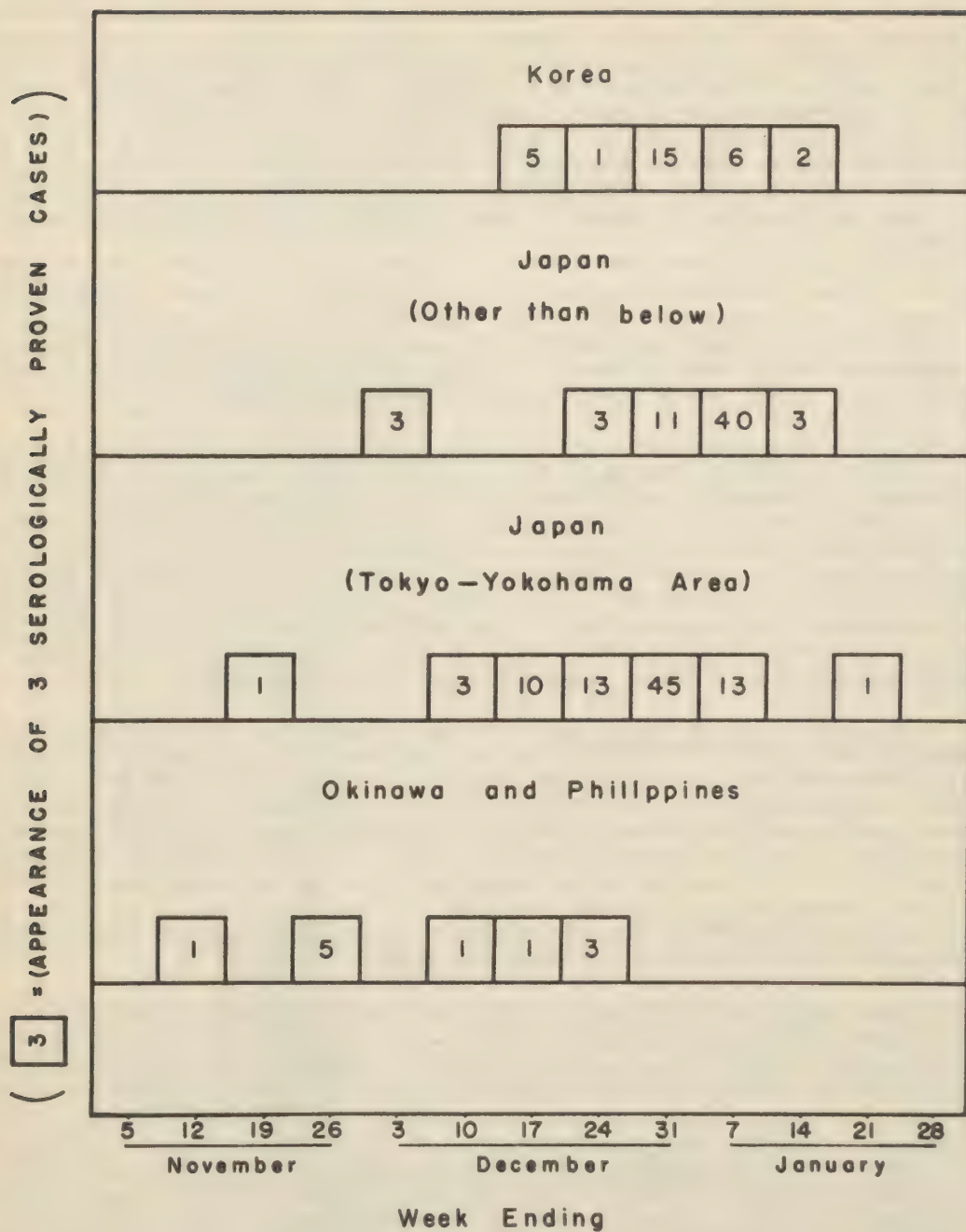


Figure 3. Appearance of Confirmed Influenza, FECOM, Winter, 1952-1953

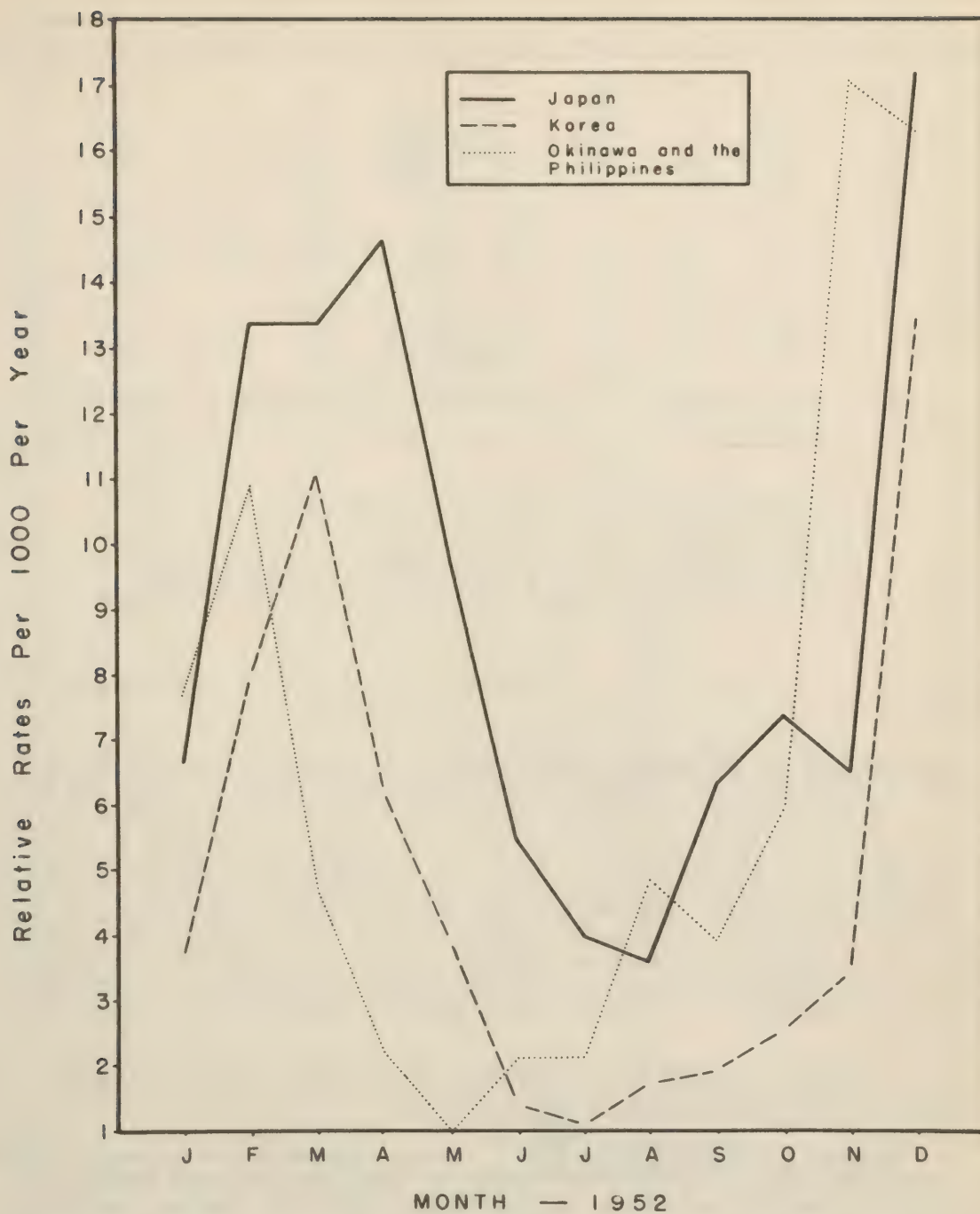


Figure 4. Upper Respiratory Disease Including Influenza, FECOM, 1952

Table 1. 1952 Laboratory Diagnosis of Influenza by Month

Month	No. of Requests	No. Paired Sera	Diag. Rises	% Positive	Diagnostic Rise				Isolation	
					PR-8	FM-1	Lee	FW-1-50 (Cuppett)	Req.	Isolates
Jan.	19	13	5	38	0	0	5		0	0
Feb.	11	8	3	37	0	1	2		1	1
Mar.	77	41	13	32	1 (1)	5	8		17	1
Apr.	71	39	14	36	1	1 (2)	13		7	0
May	20	12	4	33	0	0	4		0	0
June	8	3	0	0	0	0	0		0	0
July	5	4	0	0	0	0	0		0	0
Aug.	15	13	0	0	0	0	0		0	0
Sept.	8	6	0	0	0	0	0		0	0
Oct.	5	3	0	0	0	0	0		0	0
Nov.	17	12	2	17	0	1 (3)	0	2	0	0
Dec.	192	38	22	58	7(4)	18 (5)	0	21 (5)	61	0
TOTAL	448	192	63		9	26	32	23	86	2

- (1) This patient showed a rise to FM-1 and PR-8
- (2) This patient showed a rise to FM-1 and Lee.
- (3) This patient showed a rise to Cuppett and FM-1.
- (4) These patients showed a rise to PR-8, FM-1 and Cuppett.
- (5) Eleven of these patients showed a rise to FM-1 and Cuppett.

cases are being encountered there as evidenced by the receipt of paired sera at this laboratory.

VIRAL DISEASES OF THE CENTRAL NERVOUS SYSTEM: All cases of either clinically diagnosed or suspected viral diseases of the central nervous system in the Far East Command are reported to this section by radiogram. From these data a rough tabulation of the incidence is made. There were 25 cases during 1952 reported as non-bacterial meningitis, 116 as encephalitis, and 123 as poliomyelitis. The 25 cases grouped under the category "non-bacterial meningitis" represent such diagnoses as lymphocytic choriomeningitis, Guillain-Barre' syndrome, and acute meningitis, non-bacterial, type undetermined. Sixteen of the 25 were reported in Japan, 6 in Korea, 1 in Okinawa, and 2 in the Philippines. Because the clinical differentiation of this group of diseases and the encephalitides is often difficult, there is probably some overlapping between the two categories and certain inaccuracies in tabulation are, therefore, unavoidable.

Of the 116 cases reported as encephalitis, 32, or 27.6%, were confirmed by complement-fixation test as Japanese B encephalitis.

Japanese B Encephalitis - Figure 5, shows graphically the incidence of cases reported as encephalitis in American personnel in the Far East Command during 1952. The graph also presents those cases serologically confirmed as Japanese B encephalitis and deaths in which a presumptive diagnosis of the disease was made at autopsy. In addition to the laboratory confirmed cases shown in the graph, there were 8 Korean and 4 Okinawan natives whose sera showed diagnostic rises in titer. In contrast to the experience in 1950, relatively few cases of confirmed Japanese B encephalitis were seen in Korea during 1951 and 1952. The total number of confirmed cases of the disease for the period 1950 through 1952 is summarized below: (3, 4)

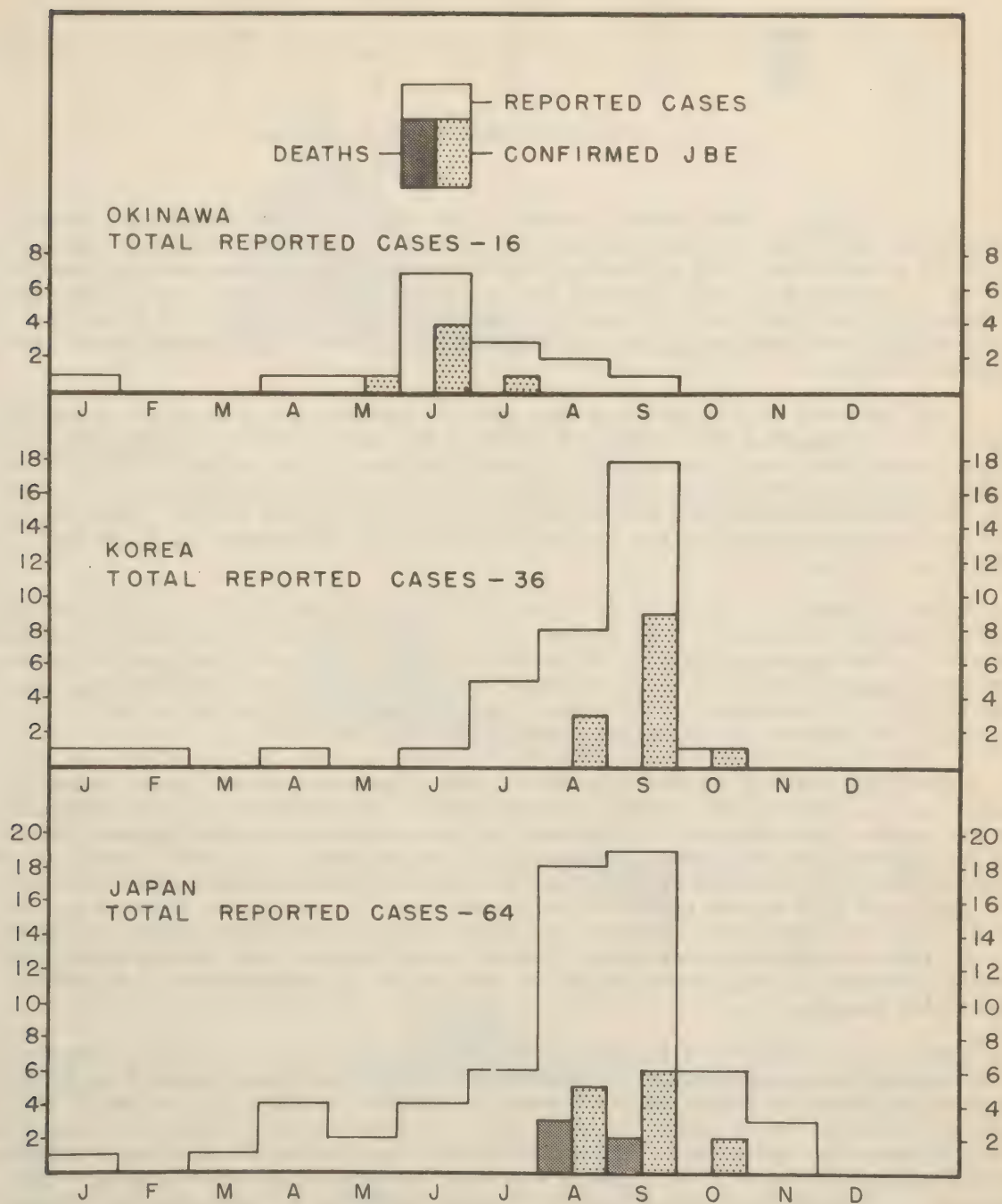


Figure 5. Encephalitis, American Personnel, FECOM, 1952

Confirmed Japanese B Encephalitis, 1950-1952

	<u>Korea</u>	<u>Japan</u>	<u>Okinawa</u>	<u>Total</u>
1950	189	10	2	201
1951	2	1	9*	12
1952	13	13	6	32

* 5 of these were Okinawan natives

Japan. There were 64 cases of encephalitis reported in U.S. personnel in 1952. Of these, 13 have been serologically confirmed as Japanese B encephalitis and 5 others died, with a presumptive diagnosis of the disease made at autopsy. The peak of incidence of both reported and confirmed cases occurred during the month of September, when there were 19 reported cases, 6 confirmed cases, and 2 deaths. All laboratory confirmed cases who contracted their disease in Japan had onset dates in August or later.

Little is known of the reservoir and mode of transmission of Japanese B encephalitis. It is suspected that certain wild birds and the mosquito Culex tritaeniorhynchus harbor the virus. Accordingly, a study was made in an attempt to determine the relationship between the relative mosquito population, the appearance of virus among the mosquito population, and the onset of viremia in wild birds. Results of these studies are reported in the section devoted to the Department of Virus and Rickettsial Diseases.

Figure 6 presents a graph of all cases in Tokyo-to reported by the Japanese Ministry of Health in the years 1948 through 1952. The criteria for serologic confirmation used by the Japanese Ministry of Health is a 4-fold rise in complement-fixation titer or a single titer of 1:16 or greater.⁽⁵⁾ Of the 711 cases reported among Japanese in 1952, 299 were confirmed by positive complement-fixation tests, and 2 by necropsy. One hundred seventy-eight deaths were reported.

Several interesting observations can be made from these data. First, there appears to be a tendency to alternate between August and September as the months of onset of cases. Secondly, it would appear that a correlation exists between the peak of incidence and the overall magnitude of the outbreak. In years of high incidence, the peak occurred earlier than in the "off" years. The epidemic years 1948, 1950, and 1952 showed a peak in incidence occurring in August, whereas in 1949 and 1951 few cases were reported in August, the peak incidence being in September. If this pattern continues these findings would suggest that for any given year a sudden increase in early August might be expected to be associated with a severe encephalitis season.

Seventy-six percent of all cases were under the age of 14. Incidence rates per 100,000 population per annum varied from a high of 30.0 in the age group 5 to 9 years (Japanese age, or approximately 4 to 8 years by Western standards) to a low of 1.0 in the age group 40 to 49 years, Japanese age. In general, case fatality rates were higher in the older age groups. The age distribution of cases of Japanese B encephalitis in Tokyo-to during 1952 suggests a greater susceptibility in the younger age groups and a relative immunity of persons beyond adolescence. Neutralizing antibodies to the virus have been demonstrated in many adults who give no history of a disease resembling encephalitis, a fact which suggests the existence of a large number of inapparent infections.^(5, 6)

Korea - Thirty-six cases of encephalitis among U.S. personnel were reported with origin in Korea. The peak of incidence occurred during the month of September, when 18 cases were reported, of which 9 were serologically confirmed. The first proven cases of the disease appeared in August, as in Japan. The total number of serologically proven cases from Korea is 13, excluding 8 Korean nationals.

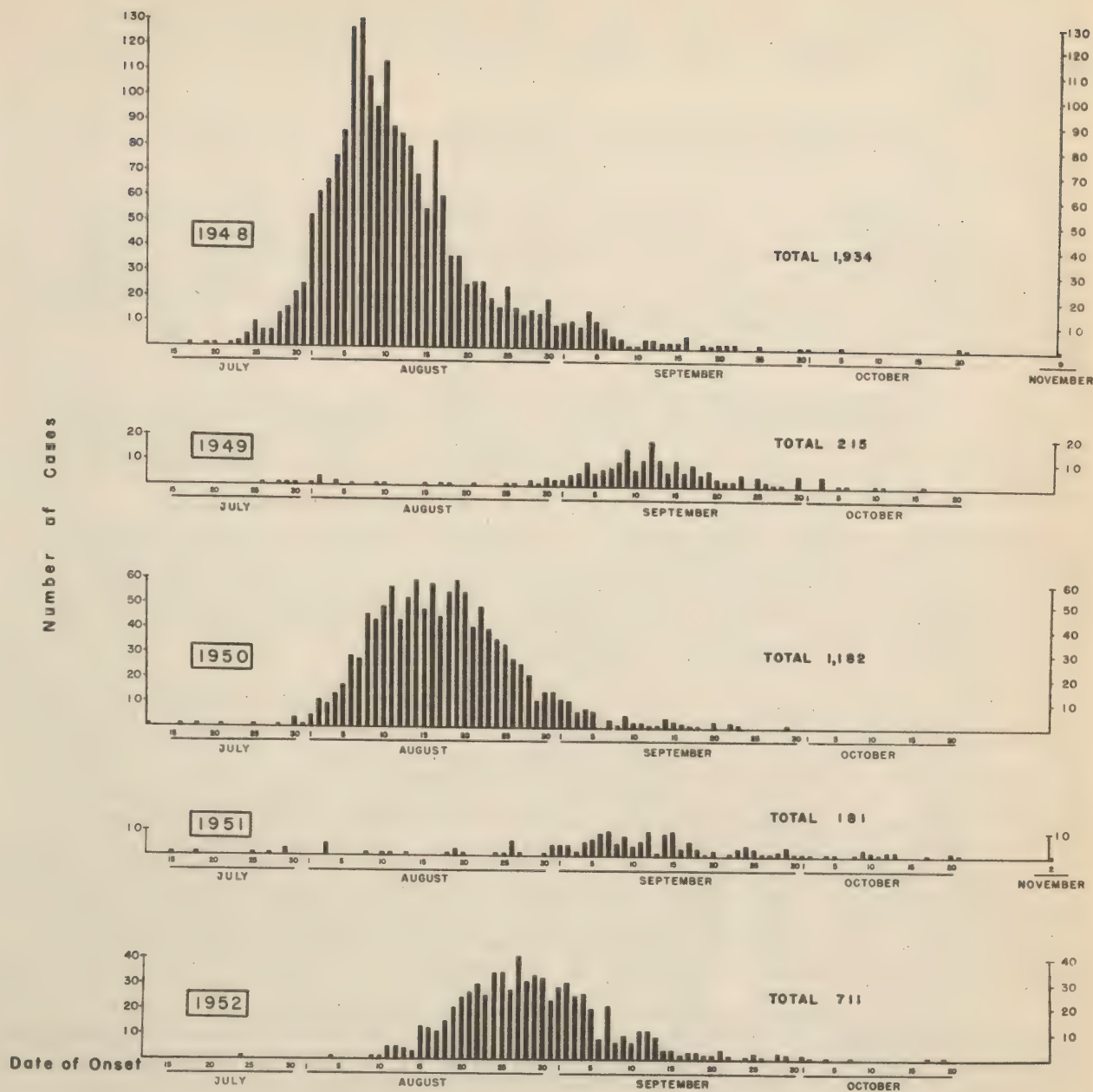


Figure 6. Japanese B Encephalitis, Tokyo-to, 1948-1952

Okinawa - A total of 16 cases of encephalitis in U.S. personnel were reported from Okinawa, of which 6 were serologically confirmed. The peak of incidence developed during June, when 7 cases were reported, 4 of which showed diagnostic rises in titer to Japanese B encephalitis. The first confirmed case developed during May, in contrast to Japan and Korea where confirmed Japanese B encephalitis did not occur until August and September. In addition, 4 Okinawan natives developed proven cases of the disease.

Philippines - No cases of encephalitis were reported among U.S. personnel in the Philippine Islands during 1952.

Poliomyelitis - The incidence of poliomyelitis in the Far East Command is shown graphically in Figure 7. One hundred twenty-three cases were reported in 1952. The greatest incidence of the disease developed in the Philippines during the months of June, July, and August. There were a total of 61 cases in the Philippines throughout the year, among which there were two deaths. The number of cases fell off sharply during the months of September and October and no cases were reported in the month of December.

Japan, Korea, and Okinawa - Thirty-four cases of poliomyelitis occurred in Japan and 25 in Korea during 1952, the peak month of incidence in both locations being July. Each area had 4 deaths from the disease. No cases have been reported from either Japan or Korea since November. Okinawa experienced only 3 cases with dates of onset during June, July, and September.

INFECTIOUS HEPATITIS: Epidemiology - Interest in infectious hepatitis was stimulated in 1950 by the high incidence of the disease in American personnel in Korea. During February 1951, the attack rate reached 35 per thousand per annum. A graph of the incidence in 1951 and 1952 in Japan, Korea, and Okinawa is shown in Figure 8. It is probable that the rates include some cases of serum hepatitis as well as infectious hepatitis as the two are difficult to distinguish clinically.

During the early spring of 1951, rates in Korea fell abruptly and throughout most of 1952, the incidence remained lower than the rate in Japan. The reason for this sudden reversal is not known. It is interesting that in February, 1951, the residual chlorine content of potable water in Korea was increased from 2 to 5 parts per million. No significance is attached to this observation. The current concept of the viral etiology of infectious hepatitis is the result of investigations begun during World War II. It is probable that several strains of viruses capable of producing infectious and serum hepatitis have evolved. Experimentally, the virus of infectious hepatitis can be transmitted to human volunteers by either the oral or parenteral administration of the feces or blood of patients in the acute phase of the disease. The virus of serum hepatitis, on the other hand, has been transmissible only via the parenteral route. The susceptibility of the agents to chlorinated water is not yet established.

As infectious hepatitis and serum hepatitis have identical clinical manifestations, it is difficult to differentiate the two. Differential diagnosis may depend solely on history of exposure, transfusion, therapeutic injections, or immunizations. The incubation period is distinctly different, and may aid in the diagnosis. Serum hepatitis develops in from 60 to 120 days, while infectious hepatitis develops in 10 to 40 days after exposure. The average incubation period of the latter disease is 25 days.

Incidence - Figure 8 shows graphically the rates per thousand per annum in Japan, Korea, and Okinawa. During 1952, less hepatitis was experienced in Korea than in the other areas of the theater. This was a sharp reversal from 1951 experience. Okinawa had the highest incidence of infection, with a peak period in May, when the rate was 15.0 per thousand per year. In Japan and Korea, minor peaks occurred in April, and again in October and November. No striking seasonal trend is evident in any command during the years 1951 and 1952.

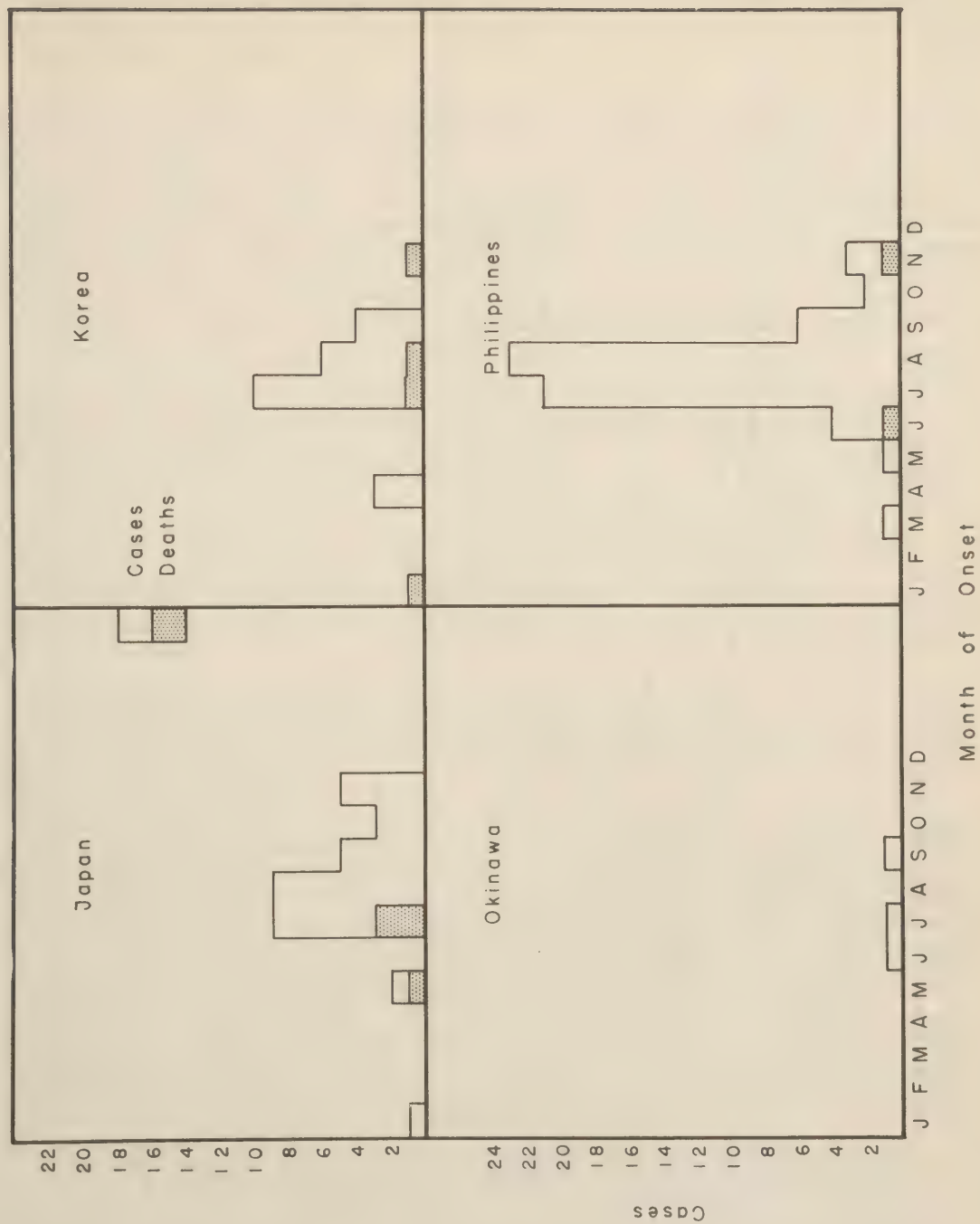


Figure 7. Poliomyelitis, American Personnel, FECOM, 1952

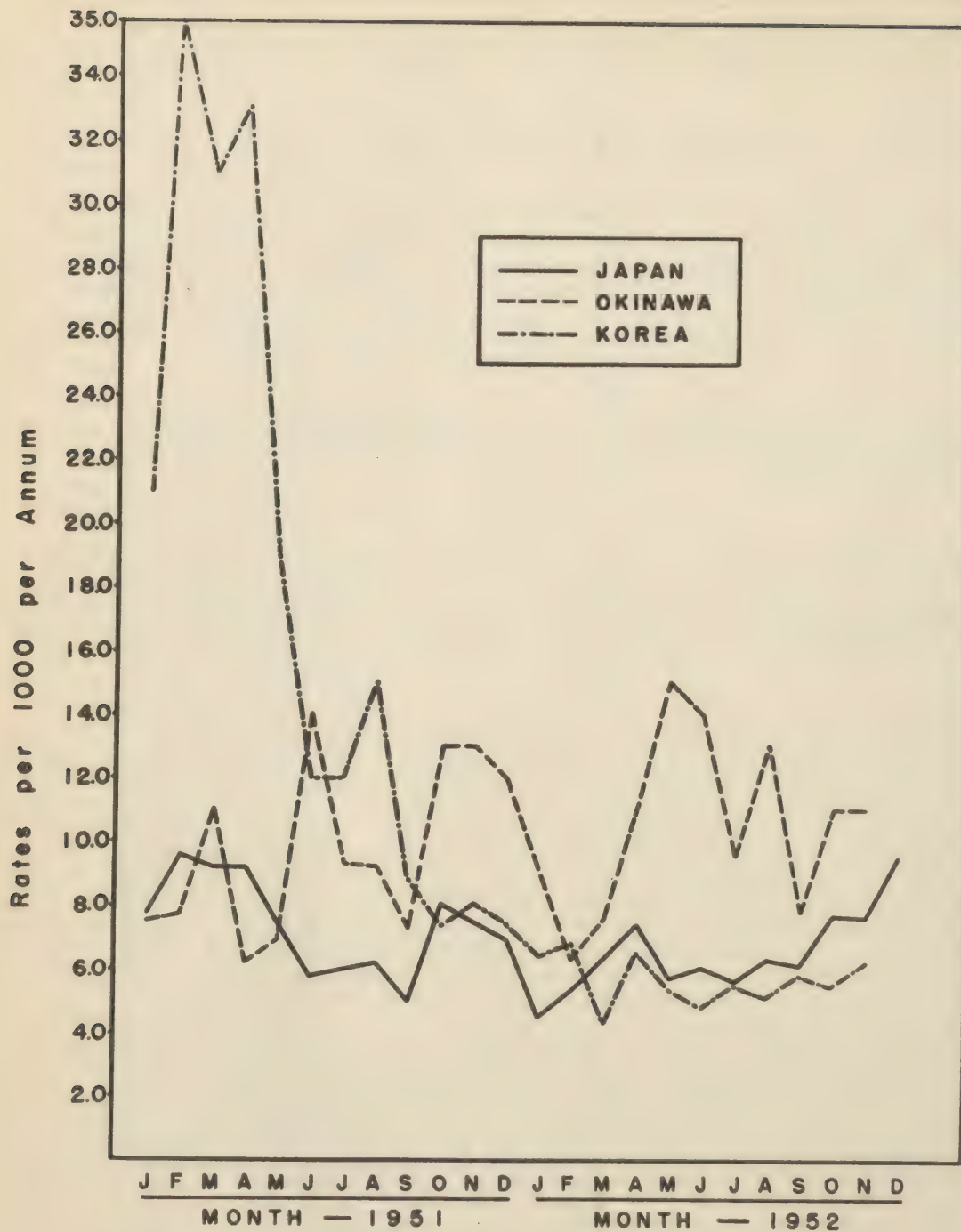


Figure 8. Hepatitis in Japan, Okinawa and Korea, all Army Personnel, 1952

Nagasaki Study - In addition to the sporadic cases which occurred throughout the theater, there was one localized outbreak of infectious hepatitis which was investigated. Between 24 February and 6 April 1952, 15 cases of infectious hepatitis were reported in persons who had stayed or eaten at the Kanko Hotel in Nagasaki, Japan, some 4 to 6 weeks prior to the onset of disease. In addition, there was one case with onset in January.

Attention was drawn to the possibility of a source of infection at the Kanko Hotel by the fact that 4 cases appeared out of a group of 7 persons who visited the hotel on the weekend of 2 and 3 February. Further search in hospitals in Japan and questioning of cases revealed that the remaining 11 patients had also stayed at the Hotel Kanko. Information was not available regarding the total number of visitors at the Kanko or any other hotel in Nagasaki during that period. Questioning of hepatitis cases in most of the military hospitals in southern Japan at this time revealed no other cases who had visited Nagasaki during the usual incubation period.

All but one of the patients developed diarrhea either while in Nagasaki or within a day after return to their respective stations. This was true of those persons who accompanied the patients to Nagasaki as well. Three naval officers who stayed at another hotel in Nagasaki on 2 and 3 February developed diarrhea but did not develop hepatitis. In no instance were enteric pathogens demonstrated on routine laboratory examination. Throughout February and early March water in the hotel was turned off from time to time. During these periods water in containers was delivered for washing and drinking. The source of this water was not known. The Nagasaki city water supply had been short and had been turned off and on for the entire city on alternate days. Many people made a practice of storing water in vessels for use during the off days. The epidemiologic pattern of the outbreak was consistent with infection from a common source at approximately the same time and it is likely, though not proven, that this source was the Kanko Hotel and most probably from a contaminated water supply.

STREPTOCOCCAL INFECTIONS: Streptococcal infections in the Far East Command have not reached sufficient proportion to be of epidemiologic concern. However, an outbreak of streptococcal sore throat appeared aboard the USS BRECKENRIDGE between 28 March and 2 April, 1952.

The clinical picture was that of a typical, acute, exudative tonsillitis or pharyngitis. Onsets could usually be timed to the hour and began with malaise, feverishness, and chilliness followed in a few hours by sore throat. In some cases sore throat was the presenting symptom. Anorexia, vomiting, and nausea were common but there was no diarrhea. Temperature ranged from 100 to 104 and the patients usually appeared acutely ill, with rapid pulse, sweating, conjunctival injection, and mild flushing of the face. Throats appeared glistening red and tonsils, when present, were usually enlarged. There was in almost all cases a patchy, purulent, non-adherent exudate over the tonsils and in more severe cases extending over the pillars. The uvula was often edematous and markedly injected. Tonsillar nodes were enlarged and tender. No instances of scarlatiniform eruption were noted. Strains of beta hemolytic streptococci isolated from the throats in the outbreak showed a pattern consistent with the Lancefield Group "A"; (see report of the Department of Bacteriology).

The USS BRECKENRIDGE, an MSTs Troop Transport, departed from San Francisco about 12 March and docked at Yokohama port on 29 March 1952. Aboard it was a crew of 510 navy personnel including 19 chief petty officers and 32 officers. There were 2,083 troop class and 363 cabin class passengers. Cabin class passengers and ship's officers ate in the cabin mess served by a galley on the same deck. Troop class passengers and enlisted crew members took their meals in the troop mess supplied by a second deck galley. Waiters and stewards ate in the cabin mess.

Between 28 March and 2 April approximately 185 cases of sore throat appeared among crew and troop passengers. The peak incidence of onset was on 30 March when

more than half the patients became ill. The distribution of cases showed certain distinct localizations. Only 3 cases could be found among persons using the cabin mess whereas 179 cases appeared among personnel eating in the troop mess. Information regarding the remaining 3 cases was not available. Since the outbreak was explosive and because of the discrepancy between the number of cases occurring among personnel who ate in the two messes, it was suspected that this represented an outbreak of food or milk-borne streptococcal sore throat arising in some manner from the troop mess. It was apparent that at the time of exposure the agent was not evenly distributed to all personnel using the troop mess. This mess served approximately 2,500 people at each meal and several containers of food and beverage were served over a 2-hour period. It was thus conceivable that only certain portions of a given menu item were contaminated. Since milk and milk products are the usual vehicle for such streptococcal outbreaks the menus for the preceding days were carefully examined.

The epidemiologic evidence pointed to re-constituted, powdered milk as a source of infection and cultures were obtained from several samples of sealed cans of this product. Although streptococci were isolated from these cans, most were Group "D" strains and they differed from organisms recovered by throat culture; (see report of the Department of Bacteriology).

It is probable that reconstituted milk was the source of infection. It seems extremely likely that this milk was contaminated after reconstitution. The milk was usually made up on the night before serving and allowed to stand at room temperature until approximately 0430, when ice was added for chilling. It was drawn off the kettles through a tap at about 0600 and taken to the mess rooms where it was distributed in pitchers. A human carrier would be the most likely source of contamination in such a case. Although this could not be definitely established, it is the explanation which seems most consistent with the observations.

DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES

The following report summarizes the significant observations made of virus and rickettsial infections in the Far East Command during the past year. This report represents the work of the permanent staff of the 406th Medical General Laboratory and the Far East Medical Research Unit. The efforts of the Commission on Hemorrhagic Fever, Armed Forces Epidemiological Board, in the search for the cause of this disease will be found in the Annual Report for the Far East Medical Research Unit.

ROUTINE EXAMINATIONS: Tables I and II present in summary form the nature and the extent of all procedures performed by this department during the past year.

Table I includes experimental procedures as well as routine serological testing. Table II indicates all recovery attempts of infectious agents from many diverse sources. In the text only those diseases of frequent occurrence, or of military importance, are discussed. For the sake of brevity, no attempt has been made to discuss infrequent observations, either positive or negative.

Japanese B Encephalitis - From the occurrence of clinical encephalitis in the Far East during the past year it is apparent that JBE virus was more widely disseminated in 1952 than in the preceding summer. (See Epidemiology Section this report). During the summer and fall of 1952, serum from 140 United Nations personnel hospitalized with a diagnosis of "encephalitis, acute, type undetermined" was received. These cases occurred in Korea (43), Japan (70), Okinawa (25), and the Philippines (2); these figures do not include cases occurring in Japanese or Korean civilians, or cases of paralytic poliomyelitis in United Nations personnel.

Because United Nations personnel were not vaccinated against JBE in 1952, and because previous vaccination history was reported on all suspected patients, both the neutralization test and the complement fixation tests were employed to obtain evidence for or against infection with JBE virus. Neutralization tests were done according to the method of Paul (1) and complement fixation tests according to the method described by Hammon (2). The diagnosis of JBE was considered

positive: if complement fixing antibodies exhibited a 4-fold rise in titer, or were sustained at 1/16 (dilution/0.25 cc serum) or greater: if an increase in serum protecting substances of 2 or more logs could be demonstrated by the neutralization test.

negative: if less than 1 log of neutralizing antibody was present in serum drawn at least ten days after onset of illness, in the absence of demonstrable complement fixing antibody.

equivocal: if levels of complement fixing antibody were not diagnostic (see above) in convalescent sera, and protection was afforded by the serum against 2 or more logs of JBE virus.

Definitive serological diagnosis, particularly of "negative", required that three serum samples from each patient be tested. Serum samples drawn in the acute phase (first 5 days) and about the 21st and 35th days of illness respectively were considered adequate for diagnosis.

Using the above criteria, sera from 78 of the 140 encephalitis suspects were considered adequate for diagnosis. Table III compares the total serological diagnoses made in 1952 with those made in 1950, an "epidemic" year.

A discussion of the geographical distribution of disease in different years will be found in the Epidemiology Section of this report. Of particular interest is the observation that in areas and seasons of high incidence, i.e. Korea, 1950, the

Table I. Serological Procedures Performed, 1952

Procedures	Neurotropic Viruses						Pneumotropic Viruses					OTHER			
	JER	SLE	WKE	EEE	RSSK	LQM	/Q Fever	Peitt IV	Infl castile	New- castle Plague	Fowl	Typhus Epid	Lepto- spira	Herpes Simplex	Total
Complement Fixation	908	13	11	5	-	34	102	52	-	-	-	10	10	6	1151
Hemagglutination In- hibition	612	-	-	-	-	-	-	-	592	28	8	-	-	6	1246
Neutralization	3419	-	10	-	11	-	-	-	-	5	5	-	-	-	3450
Virus Titration	546	2	6	2	3	-	-	-	358	8	16	-	22	19	983
Agglutination	-	-	-	-	-	-	-	-	-	-	-	-	24	-	24

Table II. Attempts at Isolation of Viruses and Rickettsiae, 1952*

	T Y P E O F M A T E R I A L											
	Tissue											
	Brain*	Spinal Fluid	Blood*	Vesicle Fluid	Viscera*	Saliva	Throat Washing	Feces	Ecto parasites	Endo-parasites	Other	Total
Human	15	27	13	2	1	1	26	8	-	-	-	93
Animal	77	2	-	-	-	-	-	18	-	-	-	97
Avian	7	-	613	-	7	-	-	-	81**	20**	-	728
Mosquito	-	-	-	-	-	-	-	-	-	-	349**	349

* Excludes attempts at recovery of the agent of Hemorrhagic Fever

** Lots or pools tested

Table III. Serologic Diagnosis of JBE: United Nations Personnel in the Far East, 1950 and 1952

	Korea /		Japan /		Okinawa /		Totals /	
	1950	1952	1950	1952	1950	1952	1950	1952
Suspects	299	43	26	70	14	25	339	140*
Adequate Sera	237	25	18	35	5	16	260	78*
Positive	189	12	10	12	2	6	201	30
Negative	38	11	6	19	0	7	44	39*
Equivocal	10	2	2	4	3	3	15	9

* Includes 2 cases from the Philippines

majority of suspects were diagnosed serologically as JBE whereas in areas and seasons of low incidence, as many as 75% of those clinically diagnosed as encephalitis (with or without adequate serum), could not be serologically confirmed as JBE. Assuming uniform sensitivity and specificity of the testing method, this suggests a dilution of suspected cases with central nervous system diseases of other etiology. A serologic study of that group of central nervous system diseases suspected of being of viral etiology but serologically negative for JBE was initiated in June of 1951, and is discussed under the heading "Aseptic Meningitis" (see following section).

The range of onset dates for this year's serologically proven cases of JBE is shown in Table IV. The earlier appearance of cases in Okinawa than in Japan and Korea is consistent with previous experience. The suggestion, that differences of latitude and climate may influence epidemic curves, should serve as a caution to interpreting data from such areas of wide latitude as all Japan. For purposes of correlating human, avian, and mosquito populations and infections, the study areas should be small and well demarcated.

Table IV. Distribution of Serologically Proven JBE Cases, 1952, By Time and Area of Infection

Area	Number of Cases	Onsets of Disease /	
		1st Case	Last Case
Korea	12	10 Aug 52	22 Sep 52
Japan	12	9 Aug 52	26 Oct 52
Okinawa	6	14 May 52	13 Jul 52
Far East	32	14 May 52	26 Oct 52

The sera of 54 Korean civilians and Korean and Chinese prisoners of war with clinical diagnoses of encephalitis were submitted for study. Of 12 patients with adequate serum sampling, eight were confirmed serologically as JBE. Two serologically proven cases of JBE occurred among Japanese civilians employed by the U.S. Army on Okinawa.

All attempts to recover JBE virus from human brain and cord (8), cerebrospinal fluid (20), and whole blood (6) were unsuccessful. Five cases in United Nations personnel terminating fatally were found to be histologically compatible with viral encephalitis. (See Pathology Department Report for 4 of these cases) No transmissible agents were recovered from the brains of these cases. All died after the 6th day of disease and the failure to isolate virus is consistent with previous experience (3). Serologic suggestion of one of these patients (R.R.) as a JBE death is based on 3.1 and 3.7 logs of neutralizing antibody in serum drawn on the 4th and 9th day of disease, plus the fact that the patient entered the Far East for

the first time 13 days prior to the onset of illness. In two other fatal cases (C.R. and M.C.) no neutralizing antibody to JBE virus was present in serum drawn 5 and 9 days after the onset of disease respectively. In neither case was there neutralizing antibody to other common viruses pathogenic to the central nervous system. Case M.C. also failed to demonstrate agglutinating antibodies to 12 live strains of leptospira.

Aseptic Meningitis - During the 18 month period beginning June, 1951 sera from a number of cases of central nervous system disease, presumably of viral origin other than JBE, were received. These cases appeared sporadically throughout the year in Japan, Korea, Okinawa, and in the Philippines. The clinical picture in well over 90% where data were available, was that of an acute febrile illness of mild to moderate severity ushered in by headache, nuchal rigidity, nausea, and lethargy. The spinal fluid in these patients showed pleocytosis, normal sugar levels, and bacterial sterility. Most cases recovered uneventfully without residua or sequelae after 1 to 6 weeks.

Clinically, these cases were variously diagnosed as encephalitis, encephalomyelitis, meningoencephalitis, nonbacterial or aseptic meningitis, lymphocytic choriomeningitis, mumps meningitis, and non-paralytic poliomyelitis. Of the 58 cases from whom adequate clinical descriptions were obtained, 9 had additional signs or symptoms of localized brain or spinal cord disease, 4 presenting evidence of pyramidal tract involvement and 5 of asymmetrical lower motor neuron involvement. Two additional cases were comatose, and 2 had a generalized convulsion at onset or during the acute phase of illness. In 3 cases there was a history of antecedent or concomitant parotitis and in 2 others there was a history of close recent contact with mumps. Five patients gave histories of preceding upper respiratory infections, 1 having an X-ray picture compatible with atypical pneumonia, and another having a severe sore throat. Two cases had histories of recent vaccination, 1 with smallpox and 1 with JBE vaccine. Three had concomitant ulcerative penile lesions. In 1 patient abdominal pain was a prominent symptom and in another retrobulbar pain. Eight of the 58 cases were under 14 years of age; the rest spread up to 40. Both sexes were represented. It appeared from the variability in clinical histories that several entities were represented by this group.

In an attempt to define etiology in these cases a series of tests were performed on available sera by this laboratory and the Army Medical Service Graduate School. The tests selected in each case were determined by clinical histories and available serum. The results of testing in 58 cases are presented in Table V. Complement fixation and/or neutralization tests were employed for the serodiagnosis of JBE, LCM, and Herpes Simplex; complement fixation and/or agglutination tests (using 12 live strains) for Leptospirosis; and complement fixation tests alone for Mumps, WEE, EEE, SLE, and Lymphogranuloma Venereum. Criteria for diagnosis were those standard at the Army Medical Service Graduate School and usually required a 4-fold rise in titer between acute and convalescent phase sera. When antibodies were demonstrated without a diagnostic rise the test result was considered equivocal.

In only 5 of the 58 cases could a positive diagnosis be made. A 4-fold rise in mumps complement fixing antibody titer was found in all of these 5 cases and was considered diagnostic of recent mumps infection. Two of these cases were children with clinical parotitis and meningitis, the other 3 were cases of aseptic meningitis with no history of parotitis or mumps contacts. Two of the equivocal cases of mumps can probably be considered positive on the basis of their clinical histories, 1 having a "painful right jaw" and the other having an upper respiratory infection with many contacts with mumps patients just prior to her illness.

One tentative diagnosis of herpes simplex meningoencephalitis was made on the basis of a rise in neutralizing antibody titer when 7 and 21 day sera were compared in egg neutralization tests; testing is incomplete as yet.

No serological evidence of leptospirosis or lymphocytic choriomeningitis (LCM) was obtained in this group of patients. This is of special interest since Beeson and Hankey (4) were able to establish leptospiral infection in 11 of 86 undiagnosed cases

of aseptic meningitis in the United States and an outbreak of central nervous system disease due to Leptospira hebdomadis B occurred among U. S. troops in Okinawa in the summer of 1949 (5).

Table V. Serodiagnosis of 58 Cases of Nonbacterial CNS Infection, 1951-1952*

	JBE	WEE	EEE	SLE	LCM	Mumps	Herpes	Psitt-LV	Leptospira
Cases Tested	48	2	2	2	47	55	44	3	49
Positive	0	0	0	0	0	5	0	0	0
Equivocal	5**	0	0	0	0	3	21**	0	1
Negative	43	2	2	2	47	47	23	3	48

* Without serological confirmation of Japanese B Encephalitis

** Neutralizing antibodies present in both acute and convalescent sera without diagnostic titer rise.

The isolation of etiologic agents from spinal fluid, blood, or stool was rarely attempted or requested in these cases and represents an important omission and area for future inquiry. This is particularly true in view of the recent implication of the Coxsackie group of viruses in the clinical syndromes of nonparalytic poliomyelitis and aseptic meningitis in the United States (6) and Scandinavia (7a, 7b).

Rabies - Rabies virus was recovered by mouse passage from 8 of 71 specimens submitted in 1952. The sources of these isolates together with the results of preliminary histopathologic examination by smear and section are shown in Table VI.

Table VI. Recovered Rabies Virus, Showing Inaccuracy of "Preliminary Diagnosis"

Accession Number	Date Received	Specimen			Preliminary Diagnosis/	
		Type	Source		By Smear	By Section
V2-20	22 Jan 52	Dog Brain	Japan		Negative	Positive
52-641	22 Apr 52	Cat Brain	Korea		-	-
52-794	23 May 52	Dog Brain	Korea		Positive	Positive
52-818	2 Jun 52	Dog Brain	Korea		Negative	Positive
52-1077	18 Aug 52	Dog Brain	Korea		Negative	-
52-1361	22 Sep 52	Dog Brain	Korea		Positive	Negative
52-1598	18 Nov 52	Dog Brain	Korea		Negative	Positive
52-1638	3 Dec 52	Dog Brain	Korea		Negative	Positive

It can be seen that the correlation between isolation of virus and the demonstration of Negri bodies in either smear or fixed tissue section was not good. Not shown in Table VI are 6 specimens reported (preliminary) as positive, 4 by smear and 2 by section.

The diagnosis of rabies by impression smear during 1952 has been unreliable and of little help to clinicians; examination of fixed tissue sections was of greater value and in only 3 cases did the results of animal inoculation fail to substantiate them. Failure to obtain better correlation is in part attributable to the condition in which most specimens originating in Korea arrived. Adequate histological examination of many specimens received was not possible because of advanced autolytic changes. The year's experience suggests that clinicians should be cautioned in the interpretation of all but final reports based upon isolation results.

Mention should be made of the identification as rabies virus of an agent submitted as the virus of JBE. Fourth passage mice, inoculated with an agent recovered from a

fatal case of "encephalitis" in Seoul were received in this laboratory. After a 6-8 day incubation period, central nervous system signs were observed in 2 of 4 mice. Negri bodies were demonstrated in the brains of these mice by smear, and fixed tissue section, as well as in mice of the 5th and 6th passage. Complement fixing antigens prepared from the brains of 5th passage mice failed to fix complement with known JBE immune serum.

Influenza - Two separate outbreaks of influenza occurred in the Far East Command during the year.

The first outbreak was relatively small, involving only a portion of the military population. Serologically proven cases first appeared in January at Sasebo among the complement of a Military Sea Transport Service vessel recently arrived from the United States. Cases appearing in Kobe and the Tokyo-Yokohama area in February were similarly confirmed serologically. Laboratory confirmation of 1 case in Korea was obtained in April. Altogether, 39 of 115 pairs of sera showed significant antibody increases. Rises to Lee virus were shown in the majority of these patients. The relative incidence of serologically confirmed cases by area of occurrence was determined more by local interest in diagnosis than by the distribution of disease.

Two strains of influenza virus were recovered from this outbreak. Both were shown to be B type viruses with an antigenic configuration (8) Ba₁, b₃. These strains were more closely related to the Warner type virus than to the Lee, and were indistinguishable from B strains recovered during the winter of 1949-1950 in the Far East Command.

The second outbreak began in November 1952, when influenza appeared in Manila. Serologic confirmation of these initial cases was obtained in early December, at about the same time the clinical entity appeared on Okinawa and in Sasebo, Japan. The disease reached the Tokyo-Yokohama area in late December, and appears at present to be widely disseminated throughout the Command. Twenty-three of 45 patients tested as of 31 December 1952 showed antibody rise to the A strains of influenza virus, with major response to the FW-1-50 (Cuppert) strain. Four transmissible hemagglutinating agents have been recovered from throat washings of acutely ill patients. These strains have been shown to be antigenically closely related to the FW-1-50 (Cuppert) strain of influenza virus. Influenza in the Far East would at this time appear to be similar to that seen at approximately the same time in other parts of the world.

It is of interest to note that the route of spread of disease in both outbreaks followed the major air transportation lines, and that in each instance 2 months or more elapsed between the time the first clinical diagnosis was made south of the island of Honshu, Japan, and the appearance in the Japan-Korea axis.

RESEARCH: Japanese B Encephalitis - Natural History - That birds play some role in the natural history of Japanese B encephalitis has been suggested by Japanese, Russian, and American investigators (9, 10a, 10b, 11), but the nature of this role has never been conclusively presented. Hammon and McClure (12) demonstrated serum neutralizing substances to JBE virus in over 20 species of Japanese wild birds. Hammon and his associates (11, 13) described viremia in American chickens, English Sparrows, House Finches, and Tricolor Blackbirds following the subcutaneous inoculation of JBE virus but did not define the immune response of laboratory infected birds. Little continuity existed between the experimental data and the information assembled from nature, and no interpretation of either findings could be made until conclusive evidence of naturally occurring bird infection could be demonstrated. To do this it would be necessary to have ready access to an avian species with a high incidence of serum protecting substances, under circumstances where the environmental ecology, particularly the mosquitoes, could be studied simultaneously.

The Study Habitat: Shin Hama Imperial Duck Refuge is a sanctuary for wild water fowl situated on Tokyo Bay, 10 miles east of central Tokyo. The closest concentration of human habitations and farmyards is 1/2 mile away. Dense bamboo thickets, effectively serving as windbreaks and screens, surround a series of ponds within a 17 acre area. During the spring and summer, the refuge abounds with nesting Black-crowned

Night Herons (BCNH) Nycticorax nycticorax, Little Egrets (LE) Egretta garzetta, and Plumed Egrets (PE) Egretta intermedia, literally thousands being concentrated in the bamboo thickets, often with less than 2 feet between individual nests. Of these 3 species, only the BCNH had any extensive contact with JBE virus, as suggested by serological survey. Twenty-five (45%) of 54 BCNH captured in previous years had neutralizing antibody to JBE virus (12). Twenty-one percent of PE had antibody, while serum neutralizing substances had never been demonstrated in LE, in spite of its close association with the other 2 species.

Detection of Avian Viremia and Immunological Response of Birds to JBE Virus: Beginning 12 May 1952 and continuing throughout the summer, nestling BCNH, LE, and PE were recovered, banded, and bled each week. These nestlings, less than 4 weeks of age, were chosen because they were easily captured and bled, and because preliminary experiments with chickens suggested that the newly hatched bird developed viremia more readily than the more mature one. (See Japanese B Encephalitis, Experimental Infections in Birds). Bleedings were done in the field by teams having no contact with laboratory virus on those days. Blood obtained from the jugular vein was inoculated intracerebrally into 8-10 gm albino mice at the time of bleeding. Inoculated mice were returned to the laboratory and observed for signs of encephalitis. Transmissible agents were presumptively identified by complement fixation tests.

Birds serving as bait in mosquito traps (see below) were bled weekly during the time the traps were in operation. Blood so obtained was similarly inoculated into mice. All birds were tested for antibody to JBE virus before being placed in traps, and again in September. For the initial testing the capacity of plasma to neutralize 320 LD₅₀ of virus was determined by an intracerebral, single dilution, neutralization test. Post-season testing was quantitative, employing 3 virus dilutions.

Mosquito Trapping: Bird-baited mosquito traps were operational in the habitat between 13 July and 14 September 1952. To determine whether mosquitoes showed a preference for certain bird species, individual traps were baited with young egrets, herons or chickens. Trapped arthropods were collected, identified, and inoculated into mice daily. Transmissible agents were presumptively identified by complement fixation tests.

From 13 July to 29 July 1952, two traps were in operation, one baited with BCNH and the other with LE. Six more traps were added on 29 July. The 8 traps were divided into 2 groups of 4, arranged on the west sides of 2 arms of a bamboo thicket. Each group was composed of 1 heron, 1 egret, 1 chicken, and 1 unbaited trap. Because by 12 August no mosquitoes had been recovered from the 2 unbaited traps, herons (BCNH) were placed in one, and egrets (LE) in the other.

The daily yield of Culex tritaeniorhynchus from each trap was divided into multiple lots with a maximum of 34 in each lot. Mosquitoes were ground in phosphate saline buffered to pH 7.6, using 0.04 cc of diluent per mosquito. All emulsions were uniform with respect to concentration of mosquito tissue, which by this technique was approximately 10% by weight. A maximum of 10 mice were inoculated with each lot; when mosquito lot size was less than 10, the number of mice inoculated was equal to the number of mosquitoes. Collected Culex pipiens were emulsified in like concentrations, lot size was determined by the number recovered from each trap. All lots were inoculated within 6 hours of collection.

Results

Recovery of Japanese B Encephalitis Virus from the Blood of Nestling Wild Birds - The numbers and frequency of bleedings, and the results of animal inoculation are indicated in Table VII. Virus was recovered 5 times from individual BCNH. Three of the 5 recovered strains produced signs of central nervous system disease and death in all of the originally inoculated mice; in the remaining 2 strains, specific morbidity was observed in 3 of 5, and 4 of 5 of the mice receiving the original inoculum. All strains required a minimum of 3 mouse passages to reduce the incubation period to 4 days or less. Two of the 5 isolates were presumptively identified by the complement

fixation test with a brain antigen prepared from the originally inoculated mice, the remainder from mice of the first passage. Cross neutralization tests for more complete identification are now in progress. Three of the 5 wild caught herons from which virus was obtained were later recovered from their nests and brought to the laboratory. These 3 birds possessed circulating antibody protecting against greater than 3 logs of JBE virus 1 month after their viremia.

It is of interest that the herons in this habitat were infected with JBE virus, and developed viremia while still in the nest. It has been shown that experimental viremia is more readily induced in newly hatched chicks than in adult chickens, (See JBE, Experimental Infections in Birds following); viremia in fledgling herons then is consistent with this observation. Viremia in the heron population was not of sporadic occurrence throughout the summer, but rather was demonstrable only between 7 and 21 August. The peak of the fledgling population, however, occurred at least four weeks earlier. Since the occurrence of viremia was not a function of bird population, it would appear that the birds acquired their infection from a vector having an interseasonal population variation.

Table VII. Occurrence of Japanese B Encephalitis Virus in the Blood Of Nestling Wild Birds - Shin Hama, Tokyo, Japan, 1952.

Date of/ Bleeding	Egret* Blood		Black-crowned Night Heron Blood	
	Tested	JBE Virus Recovered	Tested	JBE Virus Recovered
May 12	5	0	4	0
19	16	0	4	0
Jun 5	6	0	14	0
12	12	0	8	0
19	8	0	12	0
26	11	0	9	0
Jul 3	7	0	14	0
11	10	0	6	0
17	12	0	6	0
24	13	0	8	0
31	5	0	10	0
Aug 7	6	0	9	1
14	6	0	7	3
21	10	0	12	1
26	8	0	11	0
29	13	0	6	0
Sep 4	8	0	8	0
152		0	148	5

* Includes Little Egret and Plumed Egret

Recovery of Japanese B Encephalitis Virus From *C. tritaeniorhynchus* - Study of the mosquitoes in the habitat yielded significant observations with respect to:

- (1) The occurrence of bird biting mosquito species and the population changes.
- (2) The related occurrence of JBE virus in mosquitoes and birds in the same habitat.
- (3) The difference between bird species in their attractiveness to mosquitoes.

Bird-baited mosquito traps were in operation between 13 July and 14 September of 1952. Mosquitoes recovered were practically limited to 2 species, C. pipiens and C. tritaeniorhynchus, and were for the most part engorged females. Figure 1 summarizes the observations made of C. tritaeniorhynchus collected from heron (BCNH), egret (LE) and chicken-baited traps. It would appear that a marked increase in the adult C. tritaeniorhynchus population occurred about 21 July and probably was sustained at a high level until about 13 August. The population of C. pipiens appeared to be waning during the first 2 weeks of the collection period, and almost none were recovered after 1 August. These observations correlate with data obtained in 1949, 1950, and 1951 when mosquito collections were made on a much larger scale (17).

Between 2 August and 16 August virus was recovered 12 times from C. tritaeniorhynchus found in heron baited traps, and once from the same species found in egret baited traps. Since avian viremia was detected only between 6 and 21 August the recovery of virus from mosquitoes and birds was almost simultaneous. No virus was recovered from C. pipiens.

It is important to note (Figure 1) that during the period of its maximal density, C. tritaeniorhynchus exhibited distinct feeding preferences with respect to the 3 avian species serving as bait. This mosquito was recovered most frequently from heron (BCNH) baited traps; next greatest numbers were obtained from traps baited with egrets (LE), while no C. tritaeniorhynchus were recovered from chicken baited traps. This latter observation may in part be explained by the avidity and skill with which the chickens ate mosquitoes placed in their trap as a test. However, the failure of the chickens to develop viremia or antibody under the circumstances of the experiment (see below) suggests that chickens neither attracted, nor were bitten by infected mosquitoes, although herons (BCNH) a few feet away were. These preliminary observations suggest that the preferential biting habits of C. tritaeniorhynchus may determine the distribution of antibody in various species of birds (12) and may explain why some investigators have failed to demonstrate antibody in Japanese, Korean, and Okinawan chickens (14, 15, 16). Similarly, when C. pipiens were being collected, more were recovered from heron - than from egret-baited - traps.

Recovery of Japanese B Encephalitis Virus from Bait Trap Birds, and Their Immunological Response to Infection - The opportunity to demonstrate the transmission of JBE virus by naturally infected mosquitoes prompted the weekly bleeding of bait trap birds for viremia. Table VIII illustrates the temporal relationship between the occurrence of viremia in a heron and the recovery of infected mosquitoes from the same trap. Infected mosquitoes were collected from Heron Trap 2 on 2, 5, 9, 13, and 14 August. The heron (#8327) was estimated to be approximately 15 days old on 29 Jul 52 when captured and caged. Serum drawn on 1 August 1952 neutralized less than 2.0 logs LD₅₀ of JBE virus. Blood drawn on 21 August contained JBE virus, while 3 weekly bleedings prior to this date, and 2 following, did not. Serum drawn 22 September from this bird neutralized 2.5 logs LD₅₀ of virus.

While this observation was not made under controlled conditions of laboratory experiment, examination of the circumstances suggests that the only likely source of infection for this bird was the mosquito attracted to it. Transmission by other biting arthropods or endoparasites cannot be ruled out although no biting insects other than mosquitoes were recovered from the trap during its operation. The possibility of infection before caging cannot be ruled out on the basis of a neutralization test, since antibody response of birds to experimental infection may be delayed. (See JBE Experimental Infection in Birds). However, Heron #8327 was caged at least 15 days without having demonstrable viremia. This is longer than the maximal incubation period for experimental infections in the bird species thus far tested. Thus it is reasonable to assume that mosquitoes were the source of Heron #8327's infection.

Table IX summarizes the occurrence of antibody on 1 August 1952 and 22 September 1952 in those birds serving as bait throughout the 2-week period during which virus was recovered from mosquitoes (2 August through 16 August).

Table VIII. Relationship of Infected C. tritaeniorhynchus to Viremia in Black-crowned Night Herons
Heron Trap #2

7/30	31	8/1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Mosquito Isolate	0	0	0	0	0	0	0	0	0	++	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bird Isolate (A-8327)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0

Little if any antibody was present in the serum of these birds when they were placed in the traps. The exception was a young plumed egret, #8322, whose serum protected 6 of 7 mice against 320 LD₅₀ of JBE virus in a single test. Serum from the remaining birds failed to protect any of six mice against the same dose of virus. Of particular importance is the development of antibody during the course of the season in all of the BCNH, and the failure of either egrets or chickens to do so. This occurrence of antibody correlates well with the almost exclusive recovery of infected mosquitoes from heron baited traps and would strengthen the assumption that Heron #8327 was infected by mosquito bite. Together, these two observations suggest that the neutralizing substances to JBE virus in avian sera can be interpreted as evidence of previous exposure to virus, and possibly previous viremia.

Discussion

Practical knowledge of the epidemiology of an arthropod-borne disease depends upon the demonstration of relationships between reservoir and man through a vector. The explosive seasonal and geographic distribution of epidemics of encephalitis in Japan was responsible for the early concept of an arthropod vector for JBE virus. The recovery of JBE virus from C. tritaeniorhynchus, the association of the infectivity and prevalence of this mosquito with the occurrence of human and animal infection, and the failure to recover virus from any other mosquito indicate its importance as a vector. However, no satisfactory explanation for the source of infection for the mosquitoes has been advanced.

The recovery of JBE virus from the blood of the BCNH demonstrates for the first time a host in which natural infection is manifested by large amounts of blood borne virus. C. tritaeniorhynchus was feeding on this host at a time when both circulating virus in the bird and infection in the mosquito population were readily demonstrable. While infected birds may constitute a major source of mosquito infection at the peak of the mosquito population, they cannot serve as a year-round host for the virus, for viremia, when it occurs, is transient and followed by the development of antibody. Irrespective of the importance of birds to the survival of virus in nature, they are capable of producing a large pool of virus readily available to mosquitoes and may well serve as springboards to increase the absolute amount of virus in nature. It remains to be shown that birds are the only vertebrates capable of acting as intermediary hosts during the summer months. A winter host for the virus is unknown. C. tritaeniorhynchus and bird endo- and ectoparasites deserve further study to determine their potentialities in this role.

Table IX. Neutralizing Antibody to Japanese B Encephalitis Virus in
Bait Trap Birds - Shin Hama, 1952

Bird Species	Bird Number	Qualitative Neutralization	Quantitative Neutralization
		1 Aug 52	22 Sep 52
Black-crowned Night Heron (BCNH)	8276	< 2.0 logs	2.3 logs
	8308	< 2.0 logs	3.1 logs
	8327*	< 2.0 logs	2.5 logs
	8331	< 2.0 logs	2.7 logs
Egrets (LE)	8323	< 2.0 logs	< 0.5 logs
	8293	N.T.	< 0.7 logs
	8309	< 2.0 logs	< 0.7 logs
(PE)	8322	> 3.0 logs	0.7 logs
Chickens	155	< 2.0 logs	1.0 logs
	157	< 2.0 logs	< 0.6 logs
	161	< 2.0 logs	0.8 logs
	170	< 2.0 logs	< 0.6 logs

* Viremia, JBE virus demonstrated 21 Aug 52

Japanese B Encephalitis: Experimental Infection in Birds - The response of several bird species to experimental infection with JBE virus was studied to help guide and interpret the field studies reported in the foregoing section. Previously Hammon (11) demonstrated viremia of low titer in 5 of 8 two-to-three month old chickens inoculated subcutaneously with small doses of JBE virus and again (13) in 3 of 12 four-month old chickens. He was able to demonstrate little if any viremia in similarly inoculated English Sparrows, whereas House Finches and Tricolor Redwinged Blackbirds developed high titered viremia lasting several days. While only limited attempts were made to study the serological response of experimentally infected birds in Hammon's series, his data suggests that many birds, including some with viremia, had no neutralizing antibody when tested 15 to 30 days after inoculation. Kobayashi et al (19), inoculating large doses of JBE virus subcutaneously, were able to demonstrate little if any circulating virus in 10 species of Japanese wild birds, including the Black-crowned Night Heron and Teal, except for a short period immediately following inoculation. This was also their experience with 5 adult chickens. In Kobayashi's series neutralizing antibody appeared in the majority of inoculated birds in "3 weeks or later." Hammon and Kobayashi drew opposite conclusions from their respective data regarding the possibility of wild birds serving as reservoirs of virus in nature. Itoh et al (20) showed that neutralizing antibody to JBE virus experimentally induced in chickens persisted more than 6 months and concluded that if chickens were infected in nature, antibody would have been found in serum surveys of Japanese chickens. The absence of naturally occurring antibody in chickens may have explanations other than that of susceptibility, since a preliminary study of preferential biting habits of the mosquito vector has suggested that C. tritaeniorhynchus is not attracted to chickens.

The experimental studies to be reported here were designed primarily to test the importance of bird species, bird age, and dose of virus as variables influencing bird infection; to determine the periods of incubation, the durations, and the magnitudes of viremia in experimental inoculated species; and to determine the pattern of antibody response following inoculation.

Methods

Because the chicken represented a readily available and otherwise suitable bird for laboratory study, it was chosen for preliminary and more detailed observations. All chickens and chicks employed were white Japanese leghorns of the same stock. Five wild bird species (BCNH, Blue Magpie, Little Egret, Grey Starling, and Teal) were maintained in an outdoor aviary and used to study species variation after techniques had been established by chicken experiment. The problems of maintaining wild birds in captivity are discussed below under the heading "Ornithology".

JBE virus employed for inoculation and antibody assay was a strain isolated in 1951 from a pool of *C. tritaeniorhynchus* (E 311). Seventh to 12th mouse passage virus was used. With the exception of those few instances where an intravenous route was chosen, all birds were inoculated subcutaneously over the right pectoral muscles. Doses ranged from 3 to 80,000 mouse LD₅₀; all inocula were titrated in mice immediately after bird inoculation. The methods of bleeding and detection of viremia were essentially those described in the preceding section. Blood was drawn from jugular or wing veins, into heparinized syringes, and was inoculated intracerebrally into 8-10 gm albino mice. Uninoculated blood was diluted to 10⁻¹ in 10% skim milk (pH 8.3), frozen, and titrated in mice when virus was demonstrated in undiluted blood. Bleedings were performed on each bird daily or every other day after inoculation for 2 to 3 weeks. Criteria for the presence of experimentally induced viremia included:

- (1) Central nervous system signs in inoculated mice after reasonable incubation periods (3-10 days).
- (2) Death after a reasonable and similar incubation period of at least 3 of 5 mice inoculated with undiluted blood.

Confirmatory evidence was the appearance of antibody in the bird. In some cases passage and/or identification of the agent by complement fixation test was necessary.

Intracerebral neutralization tests were performed with 3 virus dilutions where possible. Plasma was tested after it was determined that heparinization did not influence titers. Criteria for the interpretation of the quantitative neutralization test were as follows:

- (1) Positive - more than 1.7 logs of virus neutralized.
- (2) Equivocal - 1.0 to 1.7 logs of virus neutralized.
- (3) Negative - less than 1.0 logs of virus neutralized.

For smaller birds, where exsanguination was to be avoided, single dilution neutralization tests were employed testing against 1.5 to 2.0 log LD₅₀ of virus. Criteria for the interpretation of the qualitative single dilution test were as follows:

- (1) Positive - at least 4 of 5 mice inoculated with virus-plasma mixture survive.
- (2) Equivocal - less than 4 of 5 mice so inoculated either die or survive.
- (3) Negative - at least 4 of 5 mice die.

Results

Experimental Infection in Chickens - Tables X and XI present the results of inoculation of adult chickens and chicks. Only 1 of 19 chickens sustained viremia following inoculation of 10 to 5,000 mouse LD₅₀ doses of JBE virus. This 6-month old chicken, 5 days after subcutaneous inoculation of 10 LD₅₀ of virus, developed low titer viremia

which persisted 3 to 4 days. Eight of 19 chickens inoculated either intravenously or subcutaneously with ten to 5,000 LD₅₀ doses had significant amounts of serum neutralizing antibody 4 to 8 weeks postinoculation.

In marked contrast were the observations made with chicks (Table XI). Of 96 chicks from 3 to 21 days of age, 75 developed readily detectable viremia. The occurrence, duration, and magnitude of viremia was independent of the dose of inoculum. The only observable effect massive inoculation had was to decrease the interval between inoculation and onset of viremia. Viremia began 3 to 48 hours after inoculation, lasted 5 to 6 days, and reached its peak 3 to 4 days after inoculation, when specific mortality was observed in mice inoculated with up to 10⁻³ dilutions of chick blood.

The antibody response was universal only in chicks developing viremia. Neutralizing antibody (2 log LD₅₀ of protection) made its appearance from 3 to 8 weeks after inoculation. This late appearance of antibody in chicks following viremia should be considered in interpreting the presence or absence of antibody in single specimens of bird sera.

An interesting observation, the significance of which is not yet clear, was made with those chicks (12%) failing to develop viremia. Seven of 45 chicks tested for antibody before inoculation by the qualitative test (Table XI) had positive (3) or equivocal (4) evidence of some serum neutralizing substance to JBE virus. Not shown in the table is that these same chicks failed to develop viremia. This observation, made on 4 separate occasions (experiments #1, 3, 4B, and 5) raises some interesting questions. Are these neutralizing substances specific antibody? Do they represent antibody passively acquired from immune hens or actively acquired in ovo? Do they persist? Does the associated "immunity" persist? Further experiments are in progress to more clearly determine the nature of these neutralizing substances.

The delay in the appearance of neutralizing antibody in inoculated chicks raised the question of whether immunity could exist in the absence of detectable antibody. In experiment 4A chicks were reinoculated during the period following viremia when antibody was still not detectable. Chicks in Experiment 4B served as controls of the same brood. Table XII summarizes the results of this experiment. The majority of chicks in Experiment 4A did not develop detectable levels of neutralizing antibody until more than 8 weeks after primary inoculation (see Table XI). It will be seen from Table XII that these chicks had a definite immunity to challenge 18 days after primary inoculation. Control chicks of Experiment 4B developed readily detectable viremia when inoculated at 3 weeks of age suggesting further that at least during the first 3 weeks of life age does not influence the susceptibility of chicks to the development of viremia. It is of some interest that although inoculated chicks during the period of viremia were caged together with uninoculated controls, the uninoculated birds failed to show viremia at 4 days, or immunity to challenge 3 weeks later. This suggests that birds, as well as mammals, acquire natural infection parenterally.

The relative sensitivities of the chick and mouse to infection was determined by parallel titrations of virus in 5-day old chicks and 8-10 gm mice. The same volume and dilution of inocula were given to mice intracerebrally and to chicks intravenously. Chicks were bled for viremia and mice were followed as with a standard virus titration. Table XIII shows the results of two such experiments. It will be seen that the sensitivity of chicks to intravenous inoculation of virus as evidenced by viremia is approximately the same as mouse sensitivity to intracerebral inoculation as evidenced by disease and mortality.

An attempt was made to determine the site of virus proliferation in the chick. In this experiment chicks were exsanguinated 3 and 5 days after inoculation and blood, intestine, spleen, and brain were titrated for infectivity. The 3rd and 5th days were chosen because virus titers in the blood could be expected to be increasing and diminishing on these days respectively. The intestine was chosen because of the predilection of another neurotropic virus, poliomyelitis, for this site; and the brain as the obvious organ of virus proliferation in mammalian JBE virus disease. As will be seen in Table XIV virus was detectable on the 3rd day in blood only. The possibility that virus was

Table X. Experimental JBE Infection in Chickens

Exp. No.	No. Of Chickens	Age	Dose	Route of Inoculation	Neutralizing "Antibody" (Qualitative)	Viremia*	Neutralizing "Antibody" (Quantitative)			
							4 wks	4-8 wks	8-12 wks	12-16 wks
I	5	> 1 yr	400 LD50	Subcutaneous	0/5	0/5	1/5	1/5	1/5	1/5
III	4	> 1 yr	5000 LD50	Subcutaneous	0/4	0/4	1/4	1/4	1(1)/4	1/5
IVA	2	> 1 yr	1000 LD50	Subcutaneous	0/2	0/2	(2)/2	2/2		(1)/4
IVB	2	> 1 yr	1000 LD50	Intravenous	0/2	0/2	(2)/2	2/2		
IVC	3	6 mos	10 LD50	Subcutaneous	0/3	1/3	(2)/3	2/3		
IVD	3	6 mos	10 LD50	Intravenous	0/3	0/3	0/3	0/3		
TOTAL	19				0/19 0%	1/19 5%	2(6)/19	8/19 40%	2(1)/9	1(1)/9

Table XI. Experimental JBE Infection in Chicks

Exp. No.	No. of Chicks	Age	Dose	Route of Inoculation	Neutralizing "Antibody" (Qualitative)	Viremia*	Neutralizing "Antibody" (Quantitative)			
							4 wks	4-8 wks	8-12 wks	12 wks
1	11	8 days	80,000 LD50	Subcutaneous	2/11	9/11	0/8	NT	NT	NT
2	3	8 days	500 LD50	Subcutaneous	NT	3/3	NT	NT	NT	NT
3	11	3 days	5 LD50	Subcutaneous	(3)/11	8/11	NT	NT	NT	NT
4A**	10	3 days	3 LD50	Subcutaneous	0/8	7/10	1/3	3/9	7/8	5/6
4B	9	21 days	8 LD50	Subcutaneous	(1)/7	7/9	1/7	7/7	5/6	NT
5	8	5 days	150 LD50	Subcutaneous	1/8	7/8	NT	NT	NT	NT
***	44	5 days	150-400 LD50	Subcutaneous	NT	34/44	NT	NT	NT	NT
TOTAL	96				3(4)/45 15%	75/96 78%	2/18 11%	10/16 62%	12/14 86%	5/6 83%

NT - Not Tested

* - Expressed as Number Positive/Number tested. Number equivocal placed in parenthesis ().

** - Chicks in Experiment 4A were reinoculated at 21 days of age (see text and Table XII). Postinoculation neutralization tests are categorized by interval following primary inoculation.

*** - This series represents the combined results of chick inoculation in 10 mosquito transmission experiments (see following section).

Table XIII. Experimentally Infected Chicks, Resistance to Challenge Inoculation

Experiment	I N O C U L A T I O N			
	Primary-Chicks Age 3 Days		Secondary-Chicks Age 21 Days	
	JBE Dose SQ	Viremia 4th Day	JBE Dose SQ	Viremia 4th Day
Chick 4A	3.2 LD ₅₀	7/10	7.9 LD ₅₀	0/10
Chick 4B	none	0/9	7.9 LD ₅₀	7/9

Table XIII. Sensitivity of Mouse and Chick to Infection with JBE Virus

		Dilution JBE Virus Inoculated				
Experiment Number		10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	10 ⁻¹⁰
IA	Chick Viremia	2/2	2/2	1/1	0/1	NT*
	Mouse Mortality	5/5	5/5	3/5	0/5	NT
IB	Chick Viremia	NT	1/3	0/4	0/4	0/2
	Mouse Mortality	5/5	5/5	0/5	0/5	NT

* Not Tested

present in high titer in such organs as liver, skin, lung, muscle or kidney remains to be determined, but the absence of virus in spleen, brain, and intestine at a time when it was readily titratable in the blood may indicate that the primary site of virus proliferation in the chick is the blood or blood forming tissues. On the 5th day virus was still readily detected in blood, and at this time made its appearance in titers as high and higher in the spleen, being again absent in brain and in intestinal mucosa. The apparent late concentration of virus in the spleen may be due to sequestration of cells containing the virus.

Experimental Infection in Japanese Wild Birds - Table XV summarizes the results of experimental inoculation of wild birds. Fifty-two birds of 5 different species ranging from 6 weeks to greater than 18 months of age were inoculated subcutaneously with doses of 5 to 600 LD₅₀ of JBE virus. Preinoculation qualitative neutralization tests were in all instances negative. Detection of viremia was attempted from blood obtained 1,3,5,7,9,11,13 and 16 days after inoculation. Viremia was established in 6 of 19 Gray Starlings (Group IA, IB, II and III) and 1 of 5 Teal; the remaining 45 birds failed to show viremia, and except for one BCNH, failed to develop antibody 4 to 8 weeks after inoculation. The viremia in Starlings and Teal began later than in the chick, appearing 5 to 7 days after inoculation. Duration of viremia was highly variable, ranging from less than 3, to more than 8 days. Of 7 Starlings in Group IA only the 2 showing viremia subsequently developed antibody. This antibody persisted for more than 8 months. Starlings in Groups IB, II, and III are currently under study for the development of antibody.

Of particular interest is the viremia shown in one Starling (Group IB). This bird had originally been given 5 LD₅₀ of JBE virus, and had failed to show either viremia or antibody response, although 2 other birds in the same series did. Inoculation with a second small dose (10 LD₅₀) 6 months after the first produced viremia which began 5 days after inoculation, and persisted longer than 8 days. This would

Table XIV. Titration of Chick Organs for Virus Content

Chick No.	Day After Inoc.	MOUSE MORTALITY BY DILUTION OF CHICK ORGAN INOCULATED											
		Blood			Spleen			Brain			Intestine		
		Undil.	10 ⁻¹	10 ⁻²	10 ⁻³	Undil.	10 ⁻¹	10 ⁻²	10 ⁻³	Undil.	10 ⁻¹	10 ⁻²	10 ⁻³
741	3	5/5	5/5	0/5	1/5	-	1/5	0/5	0/3	-	0/5	0/5	0/5
742	3	6/6	3/5	0/5	0/5	-	0/5	0/5	0/5	-	1/4	0/5	0/5
744	3	5/5	0/5	0/5	0/5	-	0/5	0/5	0/4	-	0/5	0/5	0/5
745	5	5/5	3/5	0/5	1/5	-	3/5	1/5	0/5	-	1/5	0/5	0/5
746	5	5/5	4/5	0/5	0/5	-	5/5	3/5	1/5	-	0/4	0/5	0/5
747	5	5/5	2/3	0/5	0/5	-	1/5	1/5	0/5	-	0/5	0/5	0/5
748	5	5/5	5/5	2/5	1/5	-	5/5	5/5	1/5	-	2/5	0/5	0/5

Mouse mortality considered specific

suggest that the initial failure to develop viremia was not indicative of absolute refractoriness to infection, and further suggests that inoculating in multiple small doses as probably occurs in nature, would perhaps have considerably increased the number of "takes" in the experimentally inoculated birds.

Viremia, when detected, was always of low titer. The majority of mice inoculated with undiluted blood succumbed to infection but virus was not detectable in 10⁻¹ dilutions. It should be noted that viremia was demonstrated in Starlings and Teal over one year in age and that contrary to findings in chicks and chickens, age did not appear an important variable in these limited experiments.

One might be tempted, as were Kobayashi et al (19), to conclude from the failure to induce experimental viremia regularly in presumably susceptible wild birds that these hosts could not be significant natural reservoirs of infection. It has already been shown, however, that both juvenile and adult BCNH and Blue Magpies acquire antibodies naturally in a high percentage of instances (12). Indeed (see "Natural History JBE," preceding section) JBE virus has been isolated from the blood of naturally infected nestling herons (BCNH). It would appear that certain variables may have been responsible for the apparent differences between natural and experimental infections. Questions which might be asked include the following.

(1) Does captivity with its artificial diet alter the susceptibility of wild birds to infection?

(2) Is subcutaneous inoculation inferior to intracutaneous inoculation?

(3) Would multiple small dose inocula increase the number of experimentally induced infections?

(4) Does concurrent avian disease, such as blood or intestinal parasitism, influence the development and duration of viremia?

Table XV. Experimental Infection in Wild Birds

Species	No. Birds	Age Birds	Dose in Mouse LD ₅₀ Sq	Preinoc* Neut Test	Viremia*	Postinoculation Neutralization*		
						4 weeks	8-12 weeks	12 weeks
Black-crowned Night Herons	5	6 weeks	10	0/5	0/5	1/5	1/5	1/5
Blue Magpie (Juvenile)	10	6 weeks	600	0/10	0/10	0/10		
Blue Magpie (adult)	2	> 1 year	600	0/2	0/2	0/2		
Little Egret (Juvenile)	9	2-8 weeks	10	0/9	0/9	0/9		0/9
Little Egret (Adult)	2	> 1 year	10	0/2	0/2	0/2		0/2
Gray Starling (Adult) IA	7	> 1 year	5	0/7	2/7	2/7	1/7	(2)/6
Gray Starling (Adult) IB**	7	> 18 months	10	(2)/6	1/7			
Gray Starling (Adult) II	5	> 1 year	10	0/5	0/5			
Gray Starling (Adult) III	7	> 6 months	10	0/7	3/7			
Teal (Adult)	5	> 1 year	10	0/5	1/5	0/5		0/2

* Number positive/number tested. Neutralizations are qualitative () = equivocal.
Viremia tested 3,5,7,9,11,13 and 16 days after inoculation.

** Starlings IB are same as IA but inoculated a second time.

Japanese B Encephalitis: Experimental Infection in Mosquitoes - The literature of the experimental transmission of JBE virus by mosquitoes has been reviewed by Hammon et al (21). Transmission to suckling mice by mosquitoes force fed on blood-virus suspensions has been accomplished with at least 17 different mosquito species by Japanese and American investigators. Of the few experiments designed to infect mosquitoes by feeding them on infected vertebrate hosts, all but one employed the suckling mouse as a source of virus. One investigator (22) reported a single successful transmission from chicken to suckling mouse using *C. tritaeniorhynchus*, but failed to include any data with the report. Search of the literature discloses no report of an attempt to transmit virus from bird to bird with any mosquito species. Evidence of bird to bird transmission in nature was presented in Section A but was necessarily circumstantial. It seemed desirable to establish the possibility of a bird-mosquito-bird virus cycle by laboratory experiment. The ensuing report describes 2 successful bird to mosquito to bird transmissions, some puzzling collateral observations, and some convenient techniques for the "natural" infection of mosquitoes and the study of mosquito infectivities and transmission potentials. This work was undertaken with the cooperation of the Department of Entomology; details of mosquito rearing, maintenance and feeding will be found in the report of that department. Although *C. tritaeniorhynchus* for obvious reasons was the mosquito species of choice for study, the problems of maintaining this mosquito in an indoor insectory have thus far proven insurmountable. *C. pipiens* var *pallens* was more readily maintained and was used exclusively in the following studies.

Methods

Previous work with experimental viremia in birds following subcutaneous inoculation of small amounts of virus (see preceding section) suggested the chick as an ideal laboratory host for virus in mosquito transmission experiments. Eighty-five percent of a given group of baby chicks less than 3 weeks of age could be expected to have viremia on the 3rd and 4th days following inoculation of as little as 5 mouse LD₅₀ of virus. Circulating virus in the chick host could be titrated readily at the time of mosquito feeding, and the quantity of mosquito ingested virus could be calculated from the volume of the mosquito blood meal (approximately .002 cc. - See Entomology Section). Susceptible chicks exposed to infected mosquitoes could be bled daily for the detection of circulating virus and saved for confirmatory demonstration of antibody response. In these respects the chick appeared to be a much more convenient host than the suckling mouse.

The basic plan of the transmission experiment was to inoculate several presumably susceptible chicks with 100 LD₅₀ of JBE virus. On the night of the 3rd day following inoculation, these chicks were caged individually with 100 hungry female *C. pipiens* for a period of 8-12 hours. Chicks were bled before and after proffered feeding, and their blood titrated in mice for virus content. The mean virus titer for each chick was considered an estimate of the virus content of chick blood at the time of mosquito feeding. *C. pipiens*, found unengorged after the feeding period, were discarded and the remainder maintained in separate groups at an average temperature of 80°F and a relative humidity of 70%. After the presence of viremia was established in chick hosts, mosquitoes engorged on blood containing virus were pooled. Those engorged on chicks which did not show viremia were discarded. Mosquitoes were titrated periodically for virus content (usually in lots of ten) and/or used for attempts at transmission to susceptible chicks at 7, 14, and 21 days after the initial feeding.

Results

The yield of mosquitoes feeding for the first time on infected chicks was never greater than 50%, and was usually closer to 30%. Six experiments failed at the outset either because mosquitoes failed to feed, or suffered high mortality; such failures were usually attributable to poor temperature or humidity control. One experiment failed because chicks did not develop detectable viremia at the expected time. In 3 experiments mosquitoes ingested an infected blood meal and survived in numbers appropriate for study. These will be discussed as Experiment Nos. 1, 3, and 9.

In Experiment 1, normal eight day chicks were caged overnight with mosquitoes infected 7, 14, and 21 days before with 8-20 mouse LD₅₀ of JBE virus. The following morning, the numbers of infected mosquitoes feeding upon each chick were recorded, and the chicks bitten by infected mosquitoes were bled daily for viremia. In this experiment, transmission of infection from chick to chick was successful in two instances after extrinsic incubation in the mosquito for seven days (Table XVI). Transmission was by 5 mosquitoes in each instance. Because of bacterial contamination, titration of the infectivity of the mosquitoes at the time of transmission was not possible. The blood of the chicks to which infection was transmitted, drawn 3 and 4 days after exposure, produced specific illness in all originally inoculated mice, and an antigen prepared from the brains of first passage mice fixed complement specifically with JBE immune serum. Only those chicks showing viremia after exposure to infected mosquitoes in this experiment subsequently developed significant antibody titers. Transmission was not accomplished after extrinsic incubation periods of 14 or 21 days.

In Experiment 3, although transmission was not effected at either 7 or 14 days, proof of active proliferation of virus in the mosquito following infection was obtained. Lots of mosquitoes, previously infected with 16 mouse LD₅₀ of JBE virus, were emulsified as a 10% suspension in 10% whole skim milk (pH 8.3). These emulsions were then titrated for infectivity 1, 7, and 14 days after the mosquitoes obtained an infected blood meal. The virus content per mosquito rose from a level of about 10 mouse LD₅₀ 24 hours after feeding to approximately 1300 mouse LD₅₀ at 7 days where it was maintained through the 14th day. Curiously, despite the high titer of virus in the mosquito, transmission to chicks was not accomplished in this Experiment (Table XVI). Virus was not recovered from the eggs deposited by these mosquitoes 14 days after their infection, even though virus was demonstrated on high titer in the mosquitoes. Aliquots of the eggs failed to hatch, and may not have been viable. The possibility that infection may influence mosquito fertility deserves further investigation.

In Experiment 9, mosquitoes which had fed on chicks with viremia were titrated for virus in lots of 10 at 3-day intervals through the 21st day. No attempt at transmission to chicks was made. The average virus titer in the blood of chick hosts at time of feeding was 1.7 to 2.0 logs. Assuming a 2 mgm blood meal, mosquitoes in this experiment ingested 5-10 mouse LD₅₀ of JBE virus. No virus was recovered from any mosquito lot processed from the 1st through the 21st day after feeding. It is clear that infection does not always follow the ingestion of virus by mosquitoes.

Interpretation of these 3 experiments is necessarily limited. It would seem from experiments 1 and 3 that the mosquito virus content per se bore little direct relationship to the ability of the mosquito to transmit infection. Furthermore, from Experiments 3 and 9, it will be seen that proliferation of the virus in the mosquito is not a function of exposure to virus alone, for, although the ingested dose was comparable, the virus content of mosquitoes on both 7th and 14th day in these 2 experiments was markedly different. It is clear that poorly controlled and not yet clearly defined variables may be of importance in influencing the infectability and transmission potentials of mosquitoes. Temperature and humidity were not rigidly enough controlled and may be the important variables.

The Hemagglutination Inhibition Test Applied to the Serodiagnosis of Japanese B Encephalitis - In the original publication describing the characteristics of the hemagglutinin associated with JBE virus, preliminary efforts to apply the phenomenon to the serodiagnosis of human Japanese B encephalitis were described (23). The influence of hydrogen-ion concentration upon the activity of the hemagglutinin required careful maintenance of optimal pH in the serological system. "Normal" inhibiting substances found in human serum devoid of neutralizing antibody had to be removed. Simple extraction of the serum with chloroform was shown to effectively remove or reduce "normal inhibitors" so that increases in specific hemagglutination inhibiting antibody could be demonstrated during the course of illness. The number of observations described was inadequate to correlate agglutination inhibiting antibody with neutralizing and complement fixing antibody, and test methods were not standardized. The present work

Table XVI. Transmission of JBE Virus by Infected *C. pipiens* from Chick to Chick

Extrinsic Incubation in Mosquito										
Experiment	Calculated Dose Ingested Virus	7 days			14 days			21 days		
		Virus Content per Mosquito	Mosquitoes Feeding	Trans-mission	Virus Content per Mosquito	Mosquitoes Feeding	Trans-mission	Virus Content per Mosquito	Mosquitoes Feeding	Trans-mission
1	8-20 LD 50	*	5 5 1	yes yes no	< 10 LD 50	3 2	No No	NT	1	No
3	16 LD 50	1300 LD 50	7 4 1	No No No	2500 LD 50	4	No			

* Titration unsatisfactory because of bacterial contamination

was undertaken to evaluate the usefulness of the HAI test for the sero-diagnosis of human encephalitis by a uniform test technique and to obtain, if possible, adequate data to make the necessary correlations between antibodies measured by the HAI and standard techniques.

Method

Serum from patients hospitalized with nonbacterial infections of the central nervous system in the Far East Command during the summer and fall of 1952 were used for this study. Paired or serial sera from such patients were assayed for antibody to JBE virus by the standard neutralization test (1), the complement fixation (C.F.) test (2), the hemagglutination inhibition (HAI) test. Each type of test was done by a different group of technicians. Laboratory confirmation of JBE was reported only on the basis of the two standard procedures (Routine-Japanese B Encephalitis, preceding in this report).

Preparation of the Hemagglutinating Antigen - Antigen was prepared by a protamine precipitation method (24) from mouse brains infected with JBE virus. The Nakayama strain of JBE virus, 3 passages removed from that employed in the original work in Cincinnati, was used. Brains of living infected mice were harvested aseptically at a time when at least 20% of the total inoculated group had died. These brains were washed free of blood in cold saline, ground with Alundum as a 20% suspension in saline, and refrigerated. After extraction at 4°C for 3 to 4 hours, the suspension was centrifuged at 4°C (International Pr-1 refrigerated centrifuge) at 3500 rpm for 20 minutes.

The resulting supernatant was cleared of smaller tissue aggregates by adding 5 mgm of dry protamine sulfate to each ml, and gently agitating this mixture in the refrigerator for 30 minutes. The precipitated protein was removed by centrifugation at 3500 rpm for 10 minutes. To remove the excess protamine sulfate 2 mgm of dry heparin was added to each ml of supernatant and the mixture gently agitated in the cold for 30 minutes. The protamine-heparin complex thus formed was sedimented at 3500 rpm for 10 minutes. The resulting clear pink supernatant constituted the hemagglutinating antigen. If the final supernatant was not completely transparent, an additional 0.5 mgm of heparin was added for each ml. and the mixture agitated and centrifuged as before. All antigens were stored at 4°C until used. No attempt was made to render them non-infectious for mice.

The Hemagglutination and Hemagglutination Inhibition Test - With certain exceptions, titrations of JBE hemagglutinin are made in the same manner as those for influenza virus (25). In the titration of the JBE antigen, roundbottomed Wasserman tubes, with a more shallow bowl are substituted for the more steeply sloped Kahn tubes. The influence of pH upon the JBE hemagglutinin requires that all diluents used in the serological test be buffered to pH 6.3-6.5. M/100 phosphate saline is used in place of normal saline. Chick cell suspensions (0.25%) are substituted for 0.5% Human O cells. To avoid the influence of heavy metals on the hemagglutination phenomenon, and to minimize pH changes resulting from incomplete rinsing, all glassware is chemically clean, and preferably sterile.

The purified 20% mouse brain suspension is considered a 1/5 dilution of hemagglutinin, and is titrated as influenza viruses are, dilutions and volumes employed being identical. The less concentrated cell suspension allows agglutination endpoints to be read without difficulty; the unit of JBE hemagglutinin is calculated in the same manner as that for influenza virus.

The HAI test measures the capacity of a serum, freed of normal inhibitor, to prevent the agglutination of chick red cells by 4 units of hemagglutinin. Four tenths cubic centimeter of serum, previously heated at 56°C for 30 minutes, is shaken vigorously for 5 minutes with 2.0 cc of reagent chloroform in corked Kahn tubes. The mixture is then centrifuged at 3000 rpm for 60 minutes to separate the extracted serum from the denatured protein and chloroform. The treated serum is diluted 1/10 with 2 parts of M/100 hydrochloric acid and 7 parts of buffered saline; the pH of the resultant mixture usually falls between 6.2 and 6.7. The 1/10 serum dilution is further diluted in buffered saline in 2-fold steps and 0.25 cc amounts to 1/5120. Four units of hemagglutinin contained in a 0.25 ml volume are added to each tube, and the mixture shaken. Immediately 0.5 cc of the chick cell suspension is added. Serum inhibition endpoints are read after complete sedimentation of cells, and are taken as the last dilution completely inhibiting 4 units of hemagglutinin.

Results

HAI, CF, and neutralization tests were performed on two series of patients with encephalitis. Paired or serial serum samples, of adequate volume for testing by multiple techniques, were available from 22 patients proven to have had JBE in 1952. Sera from these patients are tabulated as Series I (Tables XVII, XVIII, and XIX). Comparable sera from 25 patients suspected of encephalitis during its seasonal occurrence, but proven not to be JBE by neutralization test (Table III) were tested as Series II.

Summarized in tabular form are the relationships between antibody to JBE virus measured by the 3 test techniques in these 2 series of patients. Table XVII plots the number of individual sera with a given HAI titer against their capacity to neutralize JBE virus. It will be seen that HAI titers of 1/160 or greater were found in 42 of 54 sera (22 patients) capable of neutralizing 2 or more log LD₅₀'s of virus. The antibody titer was 1/80 or less in the 12 remaining sera in Series I; ten of these were drawn before the 10th day of disease from patients who subsequently showed HAI and CF titer rises, four-fold or greater. Two specimens, drawn 23 and 39 days after onset of disease were shown to have HAI titers of only 1/80 and 1/40 respectively, in spite of the capacity of the sera to neutralize more than 2.5 logs of virus (see S.O., Table XX). In contradistinction, HAI titers of 1/80 or less were obtained with all of the 47 sera (25 patients) which neutralized less than 1.0 log LD₅₀ of virus. Within this latter group, no significant changes in titer were seen. Thus, while it was possible in a few instances to have HAI titers less than 1/80 in sera containing 2.5 or more logs of neutralizing antibody, titers greater than 1/80 were only encountered in sera having greater than 2.0 logs LD₅₀ of neutralizing antibody.

Similar comparisons between CF and HAI antibodies are shown in Table XVIII. The patients included in Series I and II are identical with those tabulated in the preceding table. Because of individual variations in the quantities of serum received, and because of bacterial contamination or anticomplementary properties of certain specimens, the sera in the 2 tables are not identical in all instances.

Table XVII. Correlation of Neutralizing Antibody with Hemagglutination Inhibiting Antibody in Individual Sera

	Logs JBE Virus Neutralized	Serum HAI Titer, Reciprocal										
		<10	10	20	40	80	160	320	640	1280	2560	>2560
<u>SERIES I</u> Known JBE Positive	0.0 - 1.0											
	1.1 - 2.0											
	2.1 - 3.0			1*	2*	2*	2	1	2	1		
	3.1 - 4.0	1*		1*	1	3*,1	5	7	4	3		1
	4.1 - or >						5	3	5	3		
<u>SERIES II</u> Known JBE Negative	0.0 - 1.0	10	11	15	7	4						
	1.1 - 2.0											

* Serum obtained less than 10 days after onset of illness
 Series I: 54 sera representing 22 patients with proven JBE
 Series II: 47 sera representing 25 patients proven not to have JBE

Table XVIII. Correlation of Complement Fixing Antibody with Hemagglutination Inhibiting Antibody in Individual Sera

	Reciprocal CF Titer	Serum HAI Titer, Reciprocal										
		<10	10	20	40	80	160	320	640	1280	2560	>2560
<u>SERIES I</u> Known JBE Positive	< 4	1		2	2	2	5	2	5	2		
	4				1	2		5	1	1		
	8					1	2	2	4	2		1
	16						4	2	1		1	
	32							1		1		
	64									1		
	> 64								1	1		1
<u>SERIES II</u> Known JBE Negative	< 4	9	13	12	4	4						
	4											

Series I: 57 sera representing 22 patients with proven JBE
 Series II: 42 sera representing 25 patients proven not to have JBE

Table XIX. Mean Hemagglutination Inhibiting Antibody Titer by Interval of Disease

Day Of Disease	Series I* JBE Confirmed by CF Test		Series II** JBE Eliminated by Neutralization Test	
	Number Sera	Geometric Mean Titer	Number Sera	Geometric Mean Titer
0 - 10	22	152	17	17
11 - 21	13	448	18	16
22 - 35	13	640	12	17
> 35	11	480		

* 22 patients
 ** 25 patients

From Table XVII it will be seen that all but one sera with CF titers of 1/8 or greater had HAI titers of 1/160 or greater, but that HAI antibody greater than 1/80 did occur in the absence of CF antibody. The delayed and inconsistent appearance of CF antibody may be responsible for the latter observation. HAI titers of 1/80 or less were again shown in all sera of patients proven not to have JBE (Series II).

In the preceding comparisons it was implied that HAI antibody levels in the first 10 days of disease were lower than those demonstrated in sera obtained later in the illness, in spite of the fact that levels of neutralizing antibody were similar during both periods of time. The geometric mean antibody levels presented in Table XIX (Series I) tend to confirm this impression. It would appear that a trend of increasing titer during the first 35 days of disease is demonstrable. No such trend can be shown in those patients proven not to have Japanese B encephalitis.

Analysis of the serological history of the individual patients in Series I (confirmed Japanese B encephalitis) showed that 4-fold or greater increases in HAI antibody were demonstrable in 13. The various antibody changes in these 13 patients are indicated in Table XIX. All patients were found to have greater than 2.0 log LD₅₀ of neutralizing antibody, and one of them (#6, A.D.) demonstrated more than 2.4 log LD₅₀ rise of neutralizing antibody between the 4th and 18th day of disease. Significant increases (by criteria outlined in diagnostic section this report) in CF antibodies were shown in 12 of the 13 patients. Increases in HAI antibody were shown in one case as early as the 11th day of disease (#10, R1, Re). In six cases (CT, DO, FR, GB, HE, and AD) the antibody rise was detected from 12 to 33 days before confirmation of CF or neutralization test was obtained. In the remaining 7 the HAI technique demonstrated significant antibody increases at the same time as the standard tests.

It is of interest that in the 2 Orientals (#8, EF, and #13 SO) the HAI antibody never reached levels comparable to the maximal titers demonstrated in Caucasians. No explanation can be offered for this observation at the present time.

The preceding data has shown a correlation of HAI antibody with neutralizing antibody to JBE virus, and that 4-fold or greater increases in HAI antibody can be demonstrated in patients subsequently or simultaneously showing diagnostic increases in CF antibody. The HAI test by these comparisons would seem to be an extremely useful tool for the diagnosis of Japanese B encephalitis. Analysis of the data obtained from checking the sera of 78 United Nations patients listed in Table III, by the HAI technique provides greater insight into the potentialities of this technique under actual routine diagnostic circumstances. From Table XXI it will be seen that 11 of the 24 confirmed cases of encephalitis in United Nations personnel showed titer rises in HAI antibody 4-fold or greater; the remaining 13 all had sustained HAI titers greater than 1/80. Of 29 cases proved not to be JBE, 28 had maximal HAI titers less than 1/80. The remaining patient showed a rise in HAI titer from 1/80 to 1/320 when first and 21st day sera were compared. Both these sera had less than 0.6 LD₅₀ of neutralizing antibody to JBE virus. Lack of serum prevented the repetition of the HAI test. In 5 adequately studied cases in which usual laboratory techniques gave equivocal results, a significant titer rise was demonstrated twice by the HAI technique. Two more patients had titers greater than 1/80 and one of 1/80 or less. Thus, data obtained by the HAI test, and interpreted in the light of the preceding discussion, was in agreement with that obtained by standard test techniques in 57 of 58 patients; in but 1 instance would interpretation of the HAI technique have been erroneous.

In 1 fatal case of encephalitis (R.R.) where serologic diagnosis of JBE was merely suggested by standard tests, an 8-fold rise in HAI antibody from 1/40 to 1/320 was shown between 4 and 9 day sera. These sera were devoid of CF antibody, but both possessed greater than 3 logs of neutralizing antibody. Microscopically, this brain was compatible with a diagnosis of viral encephalitis. No virus was recovered from brain tissue submitted.

It would appear from the data that a 4-fold rise in HAI antibody could be interpreted with reasonable assurance as evidence of recent infection with JBE virus. Less firm is the significance of serial HAI titers greater than 1/80. It seems reasonable

Table XX. Antibody Response to JBE Virus in 13 Patients

Patient	Day Of Disease	CF Titer Reciprocal	HAI Titer Reciprocal	Logs Virus Neutralized	Patient	Day Of Disease	CF Titer Reciprocal	HAI Titer Reciprocal	Logs Virus Neutralized
#1 C.T.	6 22 38	< 4 < 4 16	40 640 -	2.4 > 4.6 -	#7 A.T.	1 21 32	< 4 32 16	20 320 160	3.5 4.3 4.3
#2 D.O.	5 21 36	< 4 < 4 16	80 640 -	2.9 2.5 2.2	#8 E.F.	6 42 56	< 4 16 16	10 160 160	3.3 4.2 3.3
#3 F.R.	2 10 21 54	< 4 - 4 16	160 80 640 -	3.7 3.1 3.7 3.1	#9 J.M.	4 22 66	4 16 64	320 2560 -	3.1 > 3.5 3.5
#4 H.B.	0 22 36	< 4 4 32	40 320 -	2.2 3.7 3.4	#10 R.L.S.	4 14 24 36	4 128 256 128	80 640 > 2560 1280	4.0 > 4.5 - 4.4
#5 G. B	6 12 22 43 61	< 4 < 4 < 4 8 8	160 640 - 640 > 2560	> 4.2 3.7 - - 3.8	#11 J.D.	3 7 30	16 - 64	320 - 1280	4.5 4.2 -
#6 A.D.	4 18	< 4 < 4	160 1280	2.4 > 4.8	#12 P.G.	5 13 31	8 - 32	80 160 1280	3.6 4.1 4.4
#13 S.O.	5 23 39 61	< 4 < 4 8 4	20 80 40 -	2.2 2.9 3.6 3.4					

Table XXI. Japanese B Encephalitis, 58 United Nations Personnel Adequately Studied by HAI Technique

24 Confirmed Positive Cases Japanese B Encephalitis

Positive by CF Test		Negative by CF		Positive by Neutralization	
Maximal HAI	HAI Titer Rise	Maximal HAI	HAI Titer Rise	Maximal HAI	HAI Titer Rise
Titer $\geq 1/80$, no titer rise	4x, or greater	Titer $\geq 1/80$, no titer rise	4x, or greater	Titer $\geq 1/80$, no titer rise	4x, or greater
13	10	0		0	1

29 Confirmed Negative Cases Japanese B Encephalitis

Maximal HAI	Maximal HAI	HAI Titer Rise
Titer $\geq 1/80$ or less	Titer $\geq 1/80$, no titer rise	4x, or greater
28	0	1

5 Equivocal Cases Japanese B Encephalitis

Maximal HAI	Maximal HAI	HAI Titer Rise
Titer $\geq 1/80$ or less	Titer $\geq 1/80$, no titer rise	4x, or greater
1	2	2

to interpret such titers as indicative of recent infection in the face of a concomitant central nervous system infection. It is not possible at this time to interpret HAI titers on single sera, without a history, as evidence for or against infection with JBE virus, since nothing is known of the persistence of the HAI antibody.

Accepting the significance of a 4-fold or greater increase in HAI titer, the HAI test would have confirmed a clinical illness of JBE in 6 of 30 (20%) patients 10 to 30 days before similar confirmation was obtained by CF or neutralization test, and would have confirmed 2 additional cases which remain in the equivocal category by standard tests. Acceptance of sustained titers of $1/160$ or greater in patients with a history of concomitant central nervous system disease would have confirmed as JBE 2 additional equivocal cases. Thus, the HAI test is potentially the most rapid method for the serodiagnosis of JBE, and when done in conjunction with CF and neutralization tests can increase the total number of diagnoses under circumstances similar to the past season's experience. Only additional testing will determine if this technique is more or less sensitive or specific than the CF and neutralization test for the diagnosis of JBE. More data is necessary to determine the persistence of HAI antibody, its relationship to inapparent infection, and the reproducibility of the HAI test in other laboratories.

Japanese B Encephalitis: Survey for Antibody Response to Vaccination, and for Inapparent Infection, 1951 - Serological observations made at the close of the 1950 outbreak of JBE in Japan and in Korea indicated that approximately 50% of all non-vaccinated American troops exposed during a single season in an endemic area of encephalitis developed neutralizing antibody to JBE virus (26) without overt evidence of disease. It seemed advisable to confirm and extend these preliminary findings by studying selected American troops during the summer of 1951 by serological methods. Men of several Army units in Japan and Korea were bled both before and after possible exposure to JBE virus. Following vaccination with standard chick embryo JBE vaccines, samples were obtained in Korea from combat troops, a rear area garrison, and men isolated upon an island. Insofar as possible samples were obtained from the same men

in October, 1951. Garrison troops in Tokyo were similarly bled except that a serum was obtained from these men prior to vaccination. These samples were supplemented by a series of specimens drawn from troops in Sapporo on the island of Hokkaido, according to a schedule identical to that of the Tokyo bleeding. The Hokkaido group, living in a reportedly nonendemic area, was intended as a control on the effect of vaccination alone upon JBE antibody levels.

Vaccination against JBE was required for all American troops in the Far East Command during the summer of 1951. The time schedule for vaccination varied considerably with the unit. In general, troops stationed in Japan received vaccine according to the recommended schedule of 1.0 cc given at 0, 7, and 30 days. Korean units, and troops entering the theater during the spring and summer of 1951, received vaccine on a schedule determined by local circumstances frequently at variance with that recommended.

Serial sera obtained were tested for neutralizing antibody to JBE virus, all sera from one individual being tested simultaneously. Sera neutralizing more than 1.7 logs of virus were considered positive, according to criteria of Paul (1).

Pertinent observations made from this survey are recorded in Table XXII. Vaccination as practiced in 1951 evoked an antibody response in 58 (16%) of an overall total of 369 individuals tested 10 to 28 days after vaccination.

In Japan, 42 (30%) of 136 individuals tested developed antibody 10-12 days after vaccination. Of the 35 positive postvaccination sera where endpoints were established there was a range of 1.8 to 3.2 logs and an average of 2.3 logs of protection demonstrated. Thirty-two of the 42 individuals in whom vaccination evoked a response were bled in the fall, 3 to 4 months after vaccination. Significant amounts of neutralizing antibody were demonstrated in 13 of these individuals.

In Korea, only 16 (7%) of 233 individuals showed significant antibody titers in postvaccination sera. Antibody persisted until the fall bleeding in nine of these 16 individuals. The discrepancy in the percentage of individuals developing antibody as a response to vaccination in the Japan (30%) and Korea (7%) groups may be attributable to less satisfactory vaccination schedules in the latter.

Table XXII indicates that only 8 of 327 individuals in which neutralizing antibody was not demonstrated in the postvaccination serum, had significant amounts of antibody in serum drawn 3 to 4 months after vaccination. The postseason sera in these 8 individuals all neutralized less than 3.0 logs LD₅₀ of JBE virus. In the light of previous experience, it seems unlikely that this represents a response to inapparent infection since it has been shown (1950) that, when antibody could be demonstrated in normal unvaccinated individuals at the end of an epidemic, it was capable of neutralizing 3.0 or more logs of virus 94% of the time.

In brief then, the 1951 survey has shown:

(1) That vaccination as practiced induced significant antibody levels in only 16% of all individuals tested and that this antibody persisted longer than 3 months in less than 10%.

(2) That faulty vaccination schedules may have reduced the efficacy of vaccination in Korea.

(3) That few, if any, Americans in Japan and in Korea experienced inapparent infection in 1951.

(4) That no neutralizing substances to JBE were detected in the sera of 144 unvaccinated individuals newly arrived in Japan.

American troops in the Far East were not vaccinated against JBE virus in 1952. Again in 1952, a paired serum survey for inapparent infection in Japan and in Korea was initiated. A plan similar to that of the preceding summer was followed; however,

Table XXII. Japanese B Encephalitis Antibody in Selected Americans,
Summer-Fall, 1951

Test Location and Troop Type	ANTIBODY RESPONSE TO VACCINATION				Spontaneous Development of Antibody	Evidence of Previous Exposure
	Immediate Response		Antibody Persistence			
	Positives	Days After Vaccination	Positives	Days After Vaccination		
<u>JAPAN:</u>						
Tokyo, Honshu	18/52	11	5/8	129	3/21	0/52
Sapporo, Hokkaido	24/94	10 - 12	8/24	90	2/73	0/92
Total	42/136(30%)		13/32		5/94	0/144
<u>KOREA:</u>						
Combat Area	5/75	24 - 28	4/5	142	2/75	-
Rear Area	6/129	21 - 28	2/6	113	1/129	-
Island Isolation	5/29	14 - 21	3/5	153	0/29	-
Total	16/233(7%)		9/16		3/233	

the intermediate (postvaccination) bleeding of 1951 was eliminated. Paired sera were drawn from 761 individuals in Japan and in Korea. Four-hundred and nine of these represented 8 U.S. Army units in different areas of Korea and 352 represented 7 U.S. Army units in different areas of Japan. These sera are currently under study and data will be reported at a later date.

The Role of Normal Inhibitor in Human Serum in the Serodiagnosis of Influenza - During the small sporadic outbreaks of influenza-like illnesses in this Command in the winter of 1951-1952, laboratory confirmation of influenza was made in a relatively small percentage of cases. Where serological evidence of influenza could not be obtained, the levels of agglutination inhibition antibody in both the acute and the convalescent sera were suggestively higher than the level of antibody in the acute phase serum of serologically proven cases. It seemed advisable to determine whether or not serum "normal inhibitors" were masking antibody rises, since an antibody rise from 1/4 to 1/64 might not be detected in serum containing a level of normal inhibitor of 1/256. If this were the case, agglutination inhibition tests on serum from which the normal inhibitor had been removed might be diagnostic. Hirst's technique of periodate digestion (27), the simplest of existing methods, was used to remove normal inhibitors.

Initial experiments with sera treated by this method were discouraging. Failure to remove normal inhibitor in this series of tests was ultimately attributed to the inactivity of a preparation, sold as sodium metaperiodate (Na IO_4) which was primarily sodium paraperiodate ($\text{Na}_3 \text{H}_2 \text{IO}_6$). The results of subsequent testing of serum digested with potassium metaperiodate (KIO_4) synthesized by the Department of Chemistry, 406th Medical General Laboratory, are summarized below.

Methods

Serum: Paired sera, submitted for serological confirmation of influenza, were used in this study. Sera from patients with respiratory disease other than influenza were tested as controls.

Agglutination Inhibition Tests: All antibody assays were done by the technique recommended by the United States Army (25). PR-8, FM-1, and Lee strains of influenza viruses were the test antigens.

Serum Digestion: Two volumes* of a saturated KIO_4 solution (solubility, 0.66 gm/100 cc) were added to 1 volume of serum previously inactivated at 56°C for 30 minutes. This mixture was incubated at 37°C for 60 minutes, following which 4 volumes of 5% dextrose in saline were added. The mixtures were allowed to react for 10 minutes at room temperature to complete the inactivation of the generated ozone. This time of incubation was considered more than adequate on the basis of the starch-iodide test for ozone which was negative within 30 seconds after the glucose-saline mixture was added. Finally, 1 volume of saline was added to bring the final serum concentration to 1:8, the serum dilution at which the agglutination inhibition tests were begun.

Results

Paired sera from 29 influenza suspects were tested against each of the 3 test viruses. In each instance, the effect of periodate digestion was controlled by a simultaneous determination on an untreated aliquot of the same serum. Of the 174 individual tests, the mean overall reduction in titer effected by periodate treatment was about 4-fold with the extremes being 0-fold and 32-fold. Periodate digestion did not render the mean serum titer for any one of the 3 test antigens significantly lower than the others.

Applying the usual criteria for the serodiagnosis of influenza, i.e. the demonstration of a 4-fold or greater rise in titer between acute and convalescent sera, 9 of the 29 suspects were positive by either or both standard and periodate techniques. Of those positive, 6 were demonstrated by both techniques, 2 by the periodate technique only, and 1 by the standard technique only. In the positive cases, mean titer reduction by periodate digestion was 4-fold in acute sera and only 2-fold in convalescent sera. Thus, a more pronounced titer rise could be demonstrated by the periodate technique in most positive cases. Table XXIII indicates the magnitude of titer rises demonstrated by the 2 techniques.

Table XXIII. Influence of Periodate Digestion of Serum Upon Magnitude Of Titer Rise in Influenza

Patient	PR-8 Virus Serum		FM-1 Virus Serum		Lee Virus Serum	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
D.A.	2	0	0	0	16	8
B.M.	2	0	2	0	> 32	4
R.M.	0	0	2	-	> 16	4
J.C.	0	0	2	0	> 8	4
I.R.	0	2	2	2	> 32	8
J.H.	0	0	2	0	> 4	0
E.C.	2	0	8	2	2	0
E.D.	> 4	2	> 32	4	0	0
W.P.	0	0	0	0	2	4

No definite statement can be made as to the validity and sensitivity of the periodate technique on the basis of one small experiment. Although routine periodate digestion for the serodiagnosis of influenza cannot yet be advocated, this study suggests that a large percentage of the agglutination inhibiting substances in human serum is "normal inhibitor" which must not be ignored when information on the absolute antibody content of individual sera is desired. The observation that the magnitude of titer rise was significantly increased by periodate treatment suggests that this technique may render the test more clearcut and accurate than the standard technique.

* Based upon solubility and molecular weights, 2 volumes of a saturated solution of KIO_4 are roughly equivalent to 0.05 M Na IO_4 recommended by Hirst for destruction of red cell receptor substance.

A Hepatitis Virus Of Mice - During the past 18 months 3 distinct hepatotropic virus diseases of albino mice have been described. The first of these was observed by Jordan and Mirick (28), and occurred in urethane treated mice 4 passages following the inoculation of liver from a fatal case of human hepatitis. This mouse disease was of slow onset, tended to chronicity, and was rarely fatal. Gledhill and Andrewes (29) demonstrated a transmissible liver disease in breeding stock of Parks strain of albino mice. This disease was 100% fatal in V.S. weanlings, with maximal death rate on the 5th day resulting from diffuse necrosis of the liver. Most recently, Nelson (30, 31) described a mouse hepatitis which, in his opinion, was distinct from each of the other two. A concise review of the characteristics of these 3 diseases, and their behavior in the laboratory, is to be found in Nelson's first publication (30). The following report describes the circumstances under which a filtrable agent causing hepatitis in mice was recovered in this laboratory, and the characteristics of this agent as they are known at the present time.

Circumstances of the Recovery of Mouse Hepatitis Virus - The present mouse disease was encountered during attempts to establish the agent of Hemorrhagic Fever in mice by serial blind passage of viscera.

One cubic centimeter of a mixture of blood and bone marrow, taken from a patient early in the course of Hemorrhagic Fever, was given intraperitoneally to each of 6 albino mice* weighing 14 to 16 grams. Nine days after inoculation, one of these mice was found dead. Advanced postmortem changes prohibited passage from this animal; however, 2 of the remaining 5 mice were sacrificed and autopsied. No obvious lesion was observed grossly. Emulsions of liver, kidney, and spleen were subinoculated into additional mice. These same viscera were passaged blindly at 7 to 9 day intervals to 6 to 8 young mice for 6 generations. All mice not sacrificed were observed a minimum of 28 days.

In the second blind passage, 1 of 3 mice alive on the 16th postinoculation day, was observed to have frank ascites, and in the succeeding 4 generations (Passages 3, 4, 5, 6) ascites, occurring in the 3rd and 4th postinoculation week occurred in the majority of mice. No disease or gross pathological change was apparent in the mice of these groups which were sacrificed on the 7th to 9th day for blind passage. Ascitic fluid was a sterile transudate, and neither it, nor liver-kidney-spleen emulsions from ascitic mice, were capable of producing similar disease upon subinoculation.

Obvious acute illness was first observed in mice of the 7th blind passage. In this passage, 4 of 14 inoculated mice were found dead on the 6th postinoculation day; of the remaining 10, one was moribund, and 6 were obviously ill. Serial intraperitoneal passage with suspensions of liver, kidney, and spleen from the sick mice quickly established that the disease was transmissible.

The Mouse Disease: While it has been possible to maintain this disease through over 40 passages in mice, the infection has been a difficult one to study quantitatively. Characteristically, only 65% (162 of 249 mice in 22 passages) of the mice inoculated intraperitoneally developed obvious signs. These mice manifested infection by a ruffled coat, "hunched" posture, lethargy, and irritability without distinct central nervous system signs. Illness appears 48 to 60 hours after inoculation. Typically, lethargy becomes more pronounced until frank prostration is evident, and once prostrate, the animal dies within 4 to 6 hours. The mortality rate in mice developing signs is approximately 90% (152/162 in 22 passages), and the majority of mice die 3 to 6 days after inoculation. When the mouse survives, the original ill defined symptoms persist for 1 to 5 days before the animal recovers its normal appearance and activity.

Pathology: Pathological changes in the viscera of infected mice appear to be similar, if not identical with, those described by Nelson (30). Striking changes,

* The albino mouse used exclusively in this laboratory at the time of recovery of the mouse hepatitis virus (M.H.V.) was a heterogeneous strain, supplied by local Japanese dealers. The parent stock of these mice was a German Swiss strain (DM) imported into Japan during the 1930's. Breeding histories and pedigrees on these mice were lost during World War II.

observed consistently in passages beyond the 10th generation, were confined to the liver, other abdominal viscera being grossly and microscopically normal in appearance. The lesion seen in mice developing symptoms 2 to 4 days after inoculation is equally well demonstrated in either living or dead animals. Livers of such mice are grossly enlarged, extremely soft, and pale yellow in color. Capsular and cut surfaces are studded with small hemorrhagic foci. The capsule is smooth and glistening, and the surface is faintly dimpled. Microscopically* in this liver, coagulation necrosis of liver cells is extremely widespread. Parenchymal cells are shrunken, stain deeply red with H & E, and contain many nuclear fragments. But few areas of the liver are spared. Mononuclear cells are observed scattered throughout the areas of necrosis, but in general cellular response is minimal. Hemorrhage into necrotic areas is not infrequent.

The liver of mice less acutely ill is large, dark, and congested. Numerous discrete gray-white foci, 1 millimeter or less in diameter, are visible over the capsular surfaces, and throughout the parenchyma. A small amount of free peritoneal fluid is occasionally seen in mice developing disease late (after the 5th day) but ascites was never a striking finding after the 7th passage. Where focal lesions are seen grossly, islands of necrosis are seen microscopically. These foci have no constant relation to lobular architecture, or blood vessels. At their peripheries a scant cellular reaction, predominantly mononuclear, is evident. Sinusoids are distended but frank hemorrhage into necrotic foci is rare.

Passage Characteristics of the M.H.V. - Experience with the mouse disease resulting from intraperitoneal inoculation suggested wide variation of mouse susceptibility. Even in the maximally susceptible weanling mice, 0.3 to 0.5 cc of infected liver suspension produced obvious disease in only 60% of the animals. Suspensions of infected liver (Passage 8) diluted beyond 1/100 and given in the same volumes, failed to kill, although clinical illness was observed sporadically in mice inoculated with dilute suspensions. When sick or normal appearing mice were sacrificed and autopsied 7 days after inoculation, the presence or absence of liver lesions did not correlate with the presence of discrete mouse illness. To determine the extent of subclinical infection, and to attempt to correlate the degree of pathological change in the liver to the occurrence of symptoms, the following experiment was devised.

Pairs of mice inoculated intraperitoneally with 0.5 cc of a 10% suspension of infected liver obtained from mice of the 10th generation, were sacrificed daily for 7 days, then at 14 and 21 days. Livers from sacrificed mice were fixed, sectioned, stained, and studied microscopically, and pooled aliquots of the same tissue were emulsified in saline (10% suspension), and subinoculated into groups of 6 mice to determine infectivity. Results of this experiment are summarized in Table XXIV.

The sampling method used in this experiment required that the mice sacrificed by day should reflect the clinical appearance of the majority of the mice. It will be seen from Table XXIV that the severity of the liver lesion progressively increased by day, presenting a picture of maximal destruction of liver tissue by the 4th and 5th postinoculation day, and that the severity of liver disease gradually diminished in those mice surviving the initial acute disease. The infectivity of liver tissue as judged from the morbidity, mortality, and incubation period was maximal on the 2nd and 4th day after inoculation, but was apparently still detectable as late as the 21st day. It is of interest to note that the mortality of the subinoculated mice correlated well with the morbidity only when the inoculum was maximally infective; and that liver suspensions possessing lesser degrees of infectivity failed to kill inoculated mice. Thus, at least for virus of the 10th generation, maximal infectivity of liver is probably attained before the appearance of obvious illness in mice,

* Tissues were sectioned, stained, and read by the Department of Pathology, 406th Medical General Laboratory.

Table XXIV. Pathology and Infectivity of Mouse Liver by Day: Mouse Hepatitis Virus

Day After Inocula- tion	Signs At Sacrifice	Mouse No.	INFECTIVITY OF LIVER			
			Microscopic Appearance of Liver	Morbidity	Mortality By 14 Days	Average Incubation Period, Days
1	No	1	Mild focal necrosis	6/6*	0/4**	6
		2	Moderate focal necrosis			
2	No	1	Minimal focal necrosis	6/6	4/4	4
		2	Moderate focal necrosis			
3	No	1	Moderate focal necrosis	6/6	5/6	4
		2	Moderate focal necrosis			
4	Yes	1	Severe diffuse necrosis	6/6	5/6	3
		2	Severe diffuse necrosis			
5	Yes	1	Severe diffuse necrosis	6/6	2/6	5
		2	Severe diffuse necrosis			
6	No	1	Moderate focal necrosis	4/6	3/6	5
		2	Moderate focal necrosis			
7	No	1	Mild focal necrosis	3/6	3/6	6
		2	Minimal focal necrosis			
14	No	1	Mild focal necrosis	2/6	0/6	6
		2	Mild focal necrosis			
21	No	1	Mild focal necrosis	1/6	0/6	7
		2	Mild focal necrosis			

* Number sick/number inoculated

** Number died/number surviving, not sacrificed

and before maximal pathological changes can be demonstrated. It would appear that discernible disease correlates better with diffuse destruction of liver tissue, than with infectivity of the liver. This experiment suggested that the most sensitive way to assess the presence or absence of infection in a given mouse was to observe its liver. Animals, with or without symptoms, could be sacrificed at 4-5 days, their livers examined grossly, and in critical experiments, the degree of liver pathology assessed microscopically.

In weanling mice, 18-21 days of age, the interval between inoculation and first evidence of disease gradually decreased from 8 days in the 9th generation to 2 days in the 23rd generation. Two-day incubation periods have been maintained through the next 30 generations.

Experiment similarly determined that the susceptibility of weanling mice varied with the dose of inoculum and the route of inoculation (Table XXV).

It will be seen that mice were as readily infected by the intracerebral route as by intraperitoneal injection, while subcutaneous or intravenous inoculation produced disease much less frequently. Gross pathological changes in the liver were seen in all sick or dead mice, irrespective of the route of inoculation, suggesting a hematogeneous dissemination of the infectious agent to the liver, at least in animals inoculated other than intraperitoneally. The response to a single intravenous inoculation was of interest because it was not as sensitive as the intraperitoneal or intracerebral routes. It was possible, therefore, that virus, given intracerebrally, was propagating in the brain,

Table XXV. Influence of Dose* and Route of Inoculation Upon Disease in Weanling Mice. 8-10 Gm.

Inoculation Route	Dose	Morbidity	Mortality	Average Incubation Period Days
Intraperitoneal	0.5 cc	6/6**	3/3***	4.3
	0.25 cc	5/6	5/5	4.3
Subcutaneous	0.5	1/6	1/6	7
Intravenous	0.25	3/6	2/6	5.3
Intracerebral	0.03 cc	5/6	2/3	4.6

* Virus 12th generation, 10% suspension of liver, kidney, and spleen

** Number sick/number inoculated

*** Number died/number surviving, not sacrificed

and secondarily involving the liver. If multiplication was occurring to any extent in central nervous system tissue, serial brain to brain passage could conceivably adapt it sufficiently to produce more regularly objective signs of infection in mice. Accordingly, brain from mice developing liver disease following intracerebral inoculation of infected liver suspensions was passaged serially brain to brain, for approximately 40 generations. Passages were made from brains of the initial mice showing symptoms of any sort in each group. All mice from the first 5 intracerebral generations were shown to have typical gross pathological changes in their livers at the time of sacrifice; indeed, the gross pathology of the intracerebrally transmitted disease was confined to the liver through 40 generations. Clinical evidence of involvement of the central nervous system was first seen after 5 passages when 1 of 9 mice was observed with paralyzed hind quarters. Frank paralysis was observed in succeeding passages only in those mice that survived the initial characteristic liver disease. The mortality rates in the intracerebral passages remained similar to those in the comparable intraperitoneal passage lines for over 30 generations. Incubation periods were the same for the 2 routes of inoculation (3-4 days) for 20 odd passages, after which it was shortened to 2 days in the intracerebral line. Irrespective of the source of inoculum (brain or liver) or the route of inoculation (IP or IC) it has never proven possible to titrate infectivity accurately, and the anticipated change in virulence by brain to brain passage failed to occur in 40 passages.

Infection in Guinea Pigs: No distinct disease was induced in 350-400 gram guinea pigs inoculated intraperitoneally with mouse liver suspensions. Guinea pig liver, 5 days after inoculation produced typical disease in mice, but not in additional guinea pigs. Guinea pig liver was similarly infectious for mice after 3 blind guinea pig passages.

Filtrability of the Infectious Agent: Two filtration experiments were done. In the first experiment, 6 mice were inoculated intraperitoneally with 0.3 cc of a 10% emulsion of infected liver, kidney, and spleen that had been filtered through a proven bacteria-tight Seitz EK pad. Six additional mice were similarly inoculated with unfiltered tissue suspensions. Two of the 6 mice inoculated with filtered suspension were ill on the 6th postinoculation day. When sacrificed, both sick mice showed typical diffuse necrosis of the liver. Subpassage of these livers produced the disease in 5 of 6 subinoculated mice. Two of 6 mice inoculated with the unfiltered suspension were ill on the 5th postinoculation day; livers of these mice exhibited the usual gross changes, and when passaged produced typical disease and lesions in 4 of 6 mice.

In the 2nd experiment, 2 of 7 mice inoculated with 1.0 cc of a 10% liver suspension filtered through a Berkfeld N candle were ill on the 4th day after inoculation. Livers of these mice were shown to have widespread focal necrosis microscopically. Five of 6 mice inoculated with an unfiltered aliquot of the same suspension developed typical disease on the 4th and 5th postinoculation day.

Infectivity of Mouse Blood and Urine - Two experiments suggest the presence of circulating virus at the time of obvious illness in mice. In the 1st experiment, 5 of 6 mice inoculated with undiluted blood from sick mice, developed typical disease and pathological findings 4 days after inoculation. A 2nd experiment, in which blood was titrated intraperitoneally, demonstrated specific illness and death by the 4th day in mice given undiluted blood. Microscopic evidence of liver involvement was present as well in mice inoculated with 10^{-2} dilutions of blood.

In one experiment, a 1/10 dilution of urine, recovered at sacrifice from 2 mice at the height of illness, produced moderate focal hepatitis in 1 of 2 mice inoculated.

Stability of Mouse Hepatitis Virus in the Frozen State - Frozen whole infected liver from the early passages (7 to 15) maintained their infectivity for at least 1 month when stored in glass at -70°C . Whole emulsions of liver or brain in saline when kept at the same temperature maintained their infectivity at least 5 months.

Sensitivity of Mouse Hepatitis Virus to Antibiotics - The use of penicillin and streptomycin in diluents to suppress the growth of bacteria which occasionally contaminated liver suspensions had little if any effect upon the infectivity of the MHV. Following the observations of Gledhill and Andrewes (29) that the mouse hepatitis described by them was prevented by prior injection of terramycin, the sensitivity of this virus to additional antibiotics was tested. Terramycin, aureomycin, and chloromycetin had little if any effect upon the experimentally induced disease, either when inoculae were incubated for 1 hour at 4°C in the presence of 2 mgm of each of the antibiotics prior to inoculation, or when mice were given a similar amount of antibiotic parenterally 3 hours before injection with infected liver suspensions.

Immunity in Mice - Only meager information is available at this time concerning the development of immunity in mice surviving initial infection. About 90% of mice surviving after demonstrating signs following initial infection failed to show evidence of infection following intraperitoneal challenge. Ten percent, however, did succumb to challenge infection. Because of the poor correlation between infection as manifested by pathological changes in the liver and clinical signs, the significance of this observation cannot be properly evaluated. No *in vitro* tests for neutralizing antibody in surviving mice have been made because of difficulty in interpreting sub-clinical infections. In 1 test no neutralizing antibody could be demonstrated in the serum of guinea pigs repeatedly inoculated with infectious mouse liver.

Attempts to Recover Mouse Hepatitis Virus from Normal Mice - Pooled livers, kidneys, and spleens from 6 randomly selected mice were inoculated intraperitoneally into 8-10 gram mice on 4 different occasions. Viscera from inoculated mice were passaged blindly at 7-10 day intervals for at least 8 generations in each instance. Mouse hepatitis virus was not recovered in these specific trials. It should be noted, however, that a liver disease of mice, not identified, was encountered in animals used in experiments to recover the agent of Hemorrhagic Fever later in the summer of 1952.

Discussion

Of considerable interest is the precise origin of this mouse hepatitis virus. Overt disease did not appear at any time in any of the originally inoculated or the first passage mice. Ascites was not apparent in 2nd or 3rd passage animals until after actual passage (blind) had been made. The acute disease subsequently reproduced in many generations did not appear until the 7th passage, and thereafter paralleled reproducible pathological changes in the liver. When fixed liver from the first seven passages was sectioned, evidence of liver involvement consistent with that seen in later passages was seen in at least 1 mouse, in most instances 2 mice of each passage. Unfortunately, tissue for sectioning in these passages were taken 15 to 23 days after inoculation, and aliquots were not passaged, whereas the tissues passaged at 7-9 days were not fixed and sectioned. Although the relationship between the pathological changes and the infectious agent was not established in early passages, both were present in mice of each passage, including the originally inoculated mice. That the original inoculum was the source of this agent could not be ruled out.

Experiment demonstrated that convalescent sera from the patient supplying the original inoculum failed to neutralize the mouse virus. Further, no evidence of neutralization of the virus, by sera from similar patients with hemorrhagic fever, could be obtained. All human sera used in these tests by Smorodintsev's criteria (32) should have contained neutralizing antibody to hemorrhagic fever virus. This suggests the mouse disease to be unrelated to hemorrhagic fever, but until definite neutralization of the M.H.V. by homologous antiserum is demonstrated, this evidence is inconclusive.

The present M.H.V. appears to be related to Nelson's virus, considered to be of mouse origin. The recovery of both agents was associated with consecutive passage of mouse viscera in mice. Both the gross and microscopic pathology of the 2 diseases are similar, if not identical. Neither is sensitive to antibiotics as was the virus of Gledhill and Andrewes. Unlike the virus of Jordan and Mirick, the disease produced by these viruses is acute, with incubation periods of less than 1 week. From his account, Nelson's virus appears to be more virulent than the presently described agent. This difference might be attributed to the mouse strains employed for the 2 viruses, since Nelson demonstrated strain differences in susceptibility to his virus. Further experiment is necessary to define more precisely the characteristics of this agent, to relate it to the hepatitis viruses described by Nelson and by Gledhill and Andrewes, and to determine the extent of infection in the mouse colony.

ORNITHOLOGY: The Aviary for Wild Birds - The need for a facility for the detention, care, and conditioning of wild birds prompted the construction of an outdoor aviary. This facility, located in Hama Park, Tokyo, within 10 minutes drive of the main laboratory, was built with the cooperation of the Japanese Imperial Government. The aviary is a compartmentalized open cage 25 x 10 x 7 feet, set upon concrete footings, and screened against insects. A central compartment 15 x 10 feet, fitted with shelving for individual small cages, is flanked by 2 end rooms of 5 x 10 feet, which serve as free flight cages. Free flight cages are provided with perches and water pools. Roofing over the entire structure is portable, reed matting and tarpaulins are being used for protection against the elements as necessary. Natural earth and sand, on a gravel base, serve as flooring.

The aviary is stocked with wild birds as they become available through netting and purchase. Table XXVI summarizes the bird species kept under aviary conditions during the past year, with their numbers and the diets upon which they were sustained.

Attempt was made to condition all birds to life in an individual cage insofar as possible. This facilitated identification of individual birds and better controlled the circumstances of diet and exercise during test. To permit closely confined birds to regain wing use and to increase their muscle tone, they were released into free flight cages at the conclusion of an experiment.

The problems of maintaining wild birds in captivity are as individual as the birds themselves. Each species has its own dietary and conditioning problems. With certain species in which interest was high because of their possible relationship to the natural history of JBE, useful information concerning these needs has been obtained, and is briefly described below.

It proved possible to take young herons and egrets from the nest 1 to 2 weeks after hatching, and to rear them in the aviary. At this age they were able to feed themselves from an offered diet. Living loaches without supplement maintained these birds during their confinement. Free in cages 5 x 10 x 7 feet, as many as 15 in a single cage thrived. Cage paralysis, observed in birds confined in small cages, did not develop in those kept in the large compartments. Under these circumstances, BCNH and Little Egrets have been successfully kept a year.

The rearing of nestling Magpies, however, required hand feeding. Young birds approximately 10 days old were fed a proprietary diet (surie) of rice and shrimp every 2 hours for 10 days. These birds usually became self-sufficient in 2 weeks, particularly when caged with older birds already adapted to aviary life. When able to feed themselves, diet was supplemented by ground meat, fruit, and bread. Free flight cages

Table XXVI. Birds Kept in Captivity: Longevity and Diet

Species	No.	Maximum Time In Captivity	No. Died	Diet	Remarks
Chinese Little Bittern (<u>Ixobrychus sinensis</u>)	2	1 1/2 weeks	2	Loaches	Dietary deficiency
Brown-eared Bulbul (<u>Ixos amaurotis</u>)	1	1 1/2 weeks	0	Raisins, surie, hamburger	Too nervous for small cage
Meadow Bunting (<u>Emberiza cioides</u>)	5	66 weeks	2	Grain, surie, bread, hamburger	Need varied diet and large cage
Rustic Bunting (<u>Emberiza rustica</u>)	10	28 weeks	9	Grain, surie	Starved on grain diet
Little Egret (<u>Egretta garzetta</u>)	25	50 weeks	12	Loaches	Need large cage
Plumed Egret (<u>Egretta intermedia</u>)	15	28 weeks	15	Loaches	Become heavily par- asitized by nematodes
Green Finch (<u>Chloris sinica</u>)	3	52 weeks	1	Grain	Do well in captivity
Blue Flycatcher (<u>Muscicapula cyanomelona</u>)	1	26 weeks	1	Surie, insects, hamburger	Diet problems
Narcissus Flycatcher (<u>Muscicapula narcissina</u>)	1	20 weeks	1	Surie, insects raisins	Diet problems
Hawfinch (<u>Coccothraustes coccothraustes</u>)	3	1 1/2 weeks	0	Grain	Need large cage, irritable
Black-crowned Night Heron (<u>Nycticorax nycticorax</u>)	30	40 weeks	10	Loaches	Need large cage
Blue Magpie (<u>Cyanopica cyanus</u>)	16	52 weeks	4	Omnivorous	Need large cage
Golden Plover (<u>Charadrius dominicus</u>)	1	22 weeks	0	Worms, grain, surie, hamburger	Needs large cage Develops sore feet
Grey Plover (<u>Squatarola squatarola</u>)	1	1 1/2 weeks	0	Hamburger, grain, worms	Needs large cage Develops sore feet
Japanese Quail (<u>Coturnix coturnix</u>)	2	12 1/2 weeks	0	Grain, surie, insects	Does well in small cage
Japanese Robin (<u>Luscinia akahige</u>)	1	5 1/2 weeks	0	Surie, raisins, hamburger	Does well in small cage
Terek Sandpiper (<u>Xenus cinereus</u>)	2	15 weeks	2	Worms, surie, hamburger	Dietetic problems
Latham's Snipe (<u>Capella hardwickii</u>)	1	19 weeks	0	Worms, hamburger	Dietetic problems Develops sore feet
Common Snipe (<u>Capella gallinago</u>)	6	7 weeks	6	Worms, hamburger	Difficult to bal- ance diet

Table XXVI. Birds Kept in Captivity: Longevity and Diet Continued

Species	No.	Maximum Time In Captivity	No. Died	Diet	Remarks
Grey Starlings (<u>Spodiopsar cineracea</u>)	19	52 weeks	2	Surie, hamburger bread, raisins	Excellent cage bird
Brown Thrush (<u>Turdus chrysolaus</u>)	3	52 weeks	1	Surie, hamburger raisins	Need large cage
Dusky Thrush (<u>Turdus maumanni</u>)	1	16 weeks	0	Surie, hamburger raisins	Need large cage
Teal (<u>Ana crecca</u>)	10	36 weeks	10	Grain	Diet problems
White Eye (<u>Zosterops palpebrosa</u>)	1	16 weeks	0	Surie, hamburger	Too small for labor- atory work

were necessary to maintain proper conditioning, and to prevent older birds from beating themselves to death.

Grey Starlings proved to be very hardy, remaining in good health for a full year while in individual cages on a diet of surie, raisins, and meat. Buntings were less hardy and developed dietary deficiencies in spite of a wide variety of foods offered. With high protein diets, Thrushes did well in small cages. All of the finches (Tree Sparrow, Hawfinch) were easily kept on grain-fruit diets, but required 1 cubic foot of cage space to maintain muscle tone. Teal and Snipe were not successfully maintained under aviary conditions.

The Shin Hama Heronry - Concurrently with the search for naturally occurring JBE virus in birds at Shin Hama a study of the bionomics of the herons in this habitat was undertaken.

The Shin Hama Imperial Duck Refuge is situated on the shore of Tokyo Bay, 10 miles from Tokyo. Five ponds, surrounded by dense bamboo thickets, are located in an area of 100 acres, surrounded on all sides by rice paddies. During all seasons of the year it is inhabited by herons of various species.

The population of a large heronry is constantly changing. Here, during the peak of summer's activity, were nearly a thousand pairs of Black-crowned Night Herons, 500 pairs each of Plumed and Little Egrets, 10 to 15 pairs of Great Egrets (Egretta alba), and Cattle Egrets (Bubeleusibis). During the winter, approximately a thousand Herons roosted each day in the bamboo, while at sundown their places were taken by a nearly equal number of Little Egrets. During the hours of their maximal activity these birds ranged over an area of at least 80 acres.

During March this population gradually changed. The Little Egrets were augmented by Plumed Egrets, until the 2 species were of nearly equal numbers. The Grey Heron left, but Cattle Egrets took their places and a few Great Egrets remained. Plumage differences indicated that there was a population shift among the BCNH as well.

Mating and nesting began in late March. Nests were built in the tops of bamboo thickets by both adults; little territorialism between species was shown in the nesting sites chosen. At peak nest density in May and June, in the choicest sites, there was a nest for each square yard of nesting surface.

Egg laying followed the pattern of nest building. First, eggs were laid in April, and peak egg production occurred in May. The average clutch of 3-4 eggs is incubated approximately 25 days before hatching. Young of all species are hatched naked, except for a soft sparse down, which in herons is brown, rather than pale yellow. Species differences in newly hatched egrets are manifested by the color of the bill, which in Little Egrets is black, in Plumed Egrets yellow. For about 5 days after hatching the young are helpless and are brooded by the hen. Between the age of 5 and 10 days they become more aware of their surroundings; at this time they first show fear, and are able to move clumsily about when danger approaches. By 10 days of age they are able to leave the nest, but do so only under stress, and move only for short distances, remaining in the foliage about the nest. Nestlings less than 2 weeks of age tolerate handling well, and are accepted by the parents after being returned to the nest. Such nestlings are readily bled from the jugular vein.

As they grow, fledglings become more venturesome and by 25 to 30 days of age are capable of leaving the nest and returning at will. By this time they are fully plumed, but incapable of sustained flight. They are recognized by their parents whether in the nest, or out, and continue to require parental feeding for at least 2 more weeks.

The mortality of embryonated eggs and nestlings is not greater than that commonly observed in other species. Greatest loss to young appears to be the result of competition in the nest, the last birds that hatch having the least chance of survival. In the usual course of events, 2 of 4 eggs laid eventually produce young which survive the fledgling stage.

Only when self-sufficiency is reached is a difference in the habits of herons and egrets conspicuous. Young egrets gradually disperse from the refuge, while the young herons continue to use Shin Hama as a roost. A steady increase of the day counted herons is noted in the thickets from May to August with peak numbers of juveniles present in late July. No such increase is noted among the juvenile egrets.

Having successfully reared one brood, the percentage of adults that return to nest again is not known. Judging by the density of late nests probably more than half raise a second brood. During the last weeks of August nesting activities rapidly comes to a close, and young had left the last nests by mid September.

Avian Population of the Kanto Plain, 1951-1952: Ten localities, within 40 miles of Tokyo, were selected as representative of the major avian habitats of the Kanto Plain. In 1951, these habitats were studied about once a week, or as often as weather and other work would permit. A total of 186 observations were made during which the numbers and species of all observed birds were tallied. In 1952, the number of observations was reduced to approximately one per month per locality, or a total of 110 tallies. Since each observation period was for at least 4 hours, approximately 750 hours in 1951 and 440 hours in 1952 were spent on these bird counts.

In 1951, 112,434 / of 149 species were recorded. In 1952, the record was 117,744 / individuals of 130 species. Because of the proximity of Tokyo Bay to the study areas, the importance of ducks in the avian picture was greatly magnified. since all ducks, except one species, are winter residents, and since no evidence linking them with JBE has been obtained, they have been eliminated from the calculations for this report. Excluding ducks, the total birds observed for 1951 and 1952 were 77,360 and 65,853, respectively. The average number of individuals tallied per species was 580 and 557, respectively. This would suggest that the bird population over the 2 years was quite similar. However, the number of birds per observation in 1951 was 415 and in 1952 it was 600. Not only did the count go up, but the impression from field observations was that there were more birds in the area in 1952 than in 1951.

Six bird species, the Grey Starling, Tree Sparrow, Plumed Egret, Little Egret, Black-crowned Night Heron, and Japanese Cormorant each made up more than 5% of the counts, cumulatively accounting for 66.4% of all birds observed. As with most numerical tallies, these figures are misleading. The 6 species are all conspicuous and

abundant and in each instance they are colonial. Considering the range of the species or its distribution over the area as well as its abundance, only 2 of these abundant species were widely distributed, the Grey Starling and the Tree Sparrow. Two other species were widely distributed and made up more than 1% of the observed population, the Meadow Bunting and the Brown-eared Bulbul.

As is usual in most avian populations, the permanent residents were numerous and widely distributed, forming the background upon which the seasonal changes of other species played. The Carrion and Jungle Crows (Corvus corone and C. leuicollis), Blue Magpie, Grey Starling, Tree Sparrow, Greenfinch, Meadow Bunting, Skylark (Alauda arvensis), Great Tit (Parus major), Long-tailed Tit (Aegithalos caudatus), Bull-headed Shrike (Lanius bucephalus), Brown-eared Bulbul, Little Egret, Black-crowned Night Heron, Spot-billed Duck (Anas poecilorhynchos), Japanese Cormorant (Phalacrocorax carbo), Turtle Dove (Streptopelia orientalis), and Kentish Plover (Choradrius alexandrinus) are considered as permanent residents. Winter residents included the Rustic Bunting, Dusky Thrush, Black-tailed Gull (Larus crossirostris), and Black-headed Gull (Larus ridibundus). Summer residents included the Great Reed Warbler (Acrocephalus arundinaceus), House Swallow (Hirundo rustica), Plumed Egret and Little Tern (Sterna albirostris). Migrants or temporary residents included the Wandering Tattler (Tringa incana), Little Stint (Calidris ruficollis), and Turnstone (Arenaria interpres).

The study of JBE virus as it was found in the Shin Hama habitat during August of 1952 suggested that Black-crowned Night Herons played a role in the dissemination of the virus in nature. While a similar role has not yet been demonstrated for other wild birds, study of widely distributed and abundant species is necessary to assess their epidemiological importance. Some of the species of potential importance that occur during the period of maximal density of C. tritaeniorhynchus are indicated in Table XXVII.

Table XXVII. Frequency of Wild Birds Potentially Important In The Natural History of Japanese B Encephalitis

Species	% Total	% Times	Antibody to JBE Virus*
	Observed Birds	Seen	
Tree Sparrow	16.6	80	15/91
Gray Starling	12.6	57	33/239
House Swallow	2.0	85	7/19
Crow, Jungle, and Carrion	0.6	70	7/83
Eastern Turtle Dove	0.6	63	3/47
Bull-headed Shrike	0.4	53	2/21

* Expressed as number having antibody to JBE virus over number tested Hammon and McClure, 1951-1952 (12).

DEPARTMENTAL ORGANIZATION: During the past year the department has been expanded to occupy approximately 4,000 square feet of the main laboratory building. This provides laboratory space, kitchen facilities, and animal rooms for the 25 to 30 individuals who comprise the permanent staff of this department. While the scope of several problems encountered in the past year has been materially influenced by the limitations of space, judicious planning and staggering of work schedules have held this problem to a minimum.

While no great emphasis has been placed upon subdivisions within the department, the professional demand and the need for concentrating facilities for certain types

of work motivated the establishment of subsections. A serological section, composed of a civilian serologist, two enlisted and two Japanese technicians, perform the majority of all serological procedures, both for the confirmation of clinical disease and for certain experimental problems. Attempts at isolation and identification of infectious agents are made by two enlisted technicians. Both these sections are administratively supervised by a medical officer whose principal responsibility is to provide definitive confirmation of the presence or absence of virus or rickettsial infection in the individual patient. This officer, through his relationship with the clinicians and laboratory officers of the Command, determines the nature and number of specimens necessary for laboratory diagnoses, decides specifically which tests to employ on the basis of the patients clinical history, and interprets and reports the results of testing to the clinician. The technical efforts of these sections are supervised by one or two additional officers as the occasion demands. Because of its distinctive mission, the ornithology section, currently composed of four individuals, is the third clearly defined division of the department. Remaining personnel are employed upon problems that vary by season and by incidence of disease and, for the most part, use facilities of the subsections as necessary.

The administrative responsibilities of the department, since they require but part-time attention, are divided among assigned officers. A veterinarian, in addition to his assigned problem, supervises the maintenance and care of laboratory animals. An officer, commissioned in the Medical Service Corps, assisted by an enlisted man, is responsible for the supply of material, animals, and equipment for this department as well as the maintenance of installed property. All assigned professional personnel contribute to the training of assigned technicians.

Mention should be made of the one major difficulty experienced with personnel for this department. Neither officers nor enlisted men, trained and experienced in virus techniques, are available through normal army replacement channels. Remarkable success has been obtained by carefully selecting interested medical officers and enlisted personnel with some basic laboratory background from the pipeline. Such officers assigned to this department in the past year have included an internist and a tissue pathologist. Similar satisfactory results have been obtained with enlisted men with backgrounds in civilian life as medical technicians. By minimizing the number of transient personnel assigned to this department, and providing for an overlap of personnel in sections, the problems usually created by the loss of trained individuals have been reduced.

DEPARTMENT OF ENTOMOLOGY

The 1952 work of the Department of Entomology embraced a great variety of activities, most of which may be grouped into two categories, namely, surveys of medically important arthropods and animal reservoirs of disease in Japan, and Japanese B encephalitis investigations. Many of the activities in the first of these categories were closely associated with hemorrhagic fever investigations in Korea, and to a considerable extent there was overlapping of what might otherwise have been considered separate activities. The relationship of various projects, and their bearing on some phases of the entomological work in Korea, will be apparent as they are discussed.

In January of the current year there was outlined an entomological research program in support of investigations of hemorrhagic fever and Japanese B encephalitis. This program not only called for specific studies with the supposed vectors and reservoirs of these two diseases, but also embraced certain necessary related activities, such as general field surveys in Korea, Japan, and Okinawa, and taxonomic studies of the ectoparasites of rodents. These related activities were designed to correct some of the existing deficiencies in our knowledge of the distribution, incidence, and biology of certain medically important arthropods, particularly those that have the ability and opportunity to transmit hemorrhagic fever, and permit detection of any existing correlation between the incidence of the disease and the abundance and distribution of possible vectors.

Limited personnel and facilities precluded implementation of the entire program outlined, and the urgency of the hemorrhagic fever studies dictated that this work be given priority. Accordingly, with the exception of studies on the biology of Culex tritaeniorhynchus, and continued efforts to colonize that species, no investigations of specific problems in the epidemiology of Japanese B encephalitis were undertaken until June. As the general survey began to delimit certain geographic areas and specific groups of arthropods as deserving more attention, ecologic and biologic studies of these groups and areas gradually developed within the broader program. The more important of these circumscribed projects were studies of the ecology of chiggers (Trombiculidae) in the Camp Fuji area, the ecology of black flies (Simuliidae) in and adjacent to Tokyo, and the incidence of biting flies in the Kyoto area.

The organization responsible for these investigations, and for the attendant supporting activities not in themselves research, consisted of the central entomological laboratory located in Tokyo, an Ecology Section located at Omiya, Saitama Prefecture, and a Taxonomic Entomology Section located at Kyoto, Kyoto Prefecture. Although the designations for the two sections indicate the primary functions carried on at those locations, there was no actual clear-cut division of activities. The locations for the sections were dictated by availability of space and qualified personnel; studies in the various subject matter fields were carried on to some extent at all three laboratories.

Those activities that are susceptible to statistical treatment are summarized in Table I. The significance of the qualifying footnotes to this table will be more apparent if the discussion pertaining to those activities is consulted.

DISTRIBUTION OF MEDICALLY IMPORTANT ARTHROPODS: The paucity of existing reliable information on the incidence and biology of medically important arthropods of Japan, other than mosquitoes, indicated the desirability of extending our knowledge in this general field. It was deemed particularly important to assemble more detailed data concerning the occurrence of actual or potential animal reservoirs and vectors of human diseases in the vicinity of bivouac and maneuver areas. Plans were therefore laid for surveys to be conducted in geographically and ecologically representative areas of Japan at various seasons of the year. Although these plans could not be carried out with the desired degree of thoroughness, the general surveys accomplished nevertheless produced a great deal of information of value both with respect to specific problems and to the general knowledge of the field. Survey activities were carried out in two ways. Almost daily field work involving survey work of some sort was accomplished in the vicinity of some or all of the three bases of operation, i.e.

Table I. Statistical Summary of Activities

Collections of Arthropods and Animal Hosts

Mosquitoes (Culicidae)

Adults	35,610
Larvae and pupae	120,000 (1)

Black Flies (Simuliidae)

Adults	317
Larvae and pupae	2,777

Miscellaneous Diptera	10,435
-----------------------------	--------

Ectoparasites (Acarina, Anoplura, Siphonaptera)	12,000 (2)
---	------------

Small Mammals	1,313
---------------------	-------

Preparations for Study and Permanent References

Slide mounts	15,192
Pinned insects	3,535
Illustrations (species)	103 (3)
Black fly rearings	1,747
Leptospira cultures	156
Animal skins and skulls	440

Identification of Arthropods and Animal Hosts

Mosquitoes (Culicidae)

Adults	34,692
Larvae	500 (4)

Black flies (Simuliidae)	913 (5)
--------------------------------	---------

Miscellaneous Diptera	12,595
-----------------------------	--------

Ectoparasites (Acarina, Anoplura, Siphonaptera)	20,000 (2)
---	------------

Small Animals	1,437
---------------------	-------

Leptospira cultures	10
---------------------------	----

Transmittal of Reference Collections of Biological Material

Acarina and Siphonaptera, slide preparations

Army Medical Service Graduate School	185
British Museum (Natural History), England	94
California Academy of Sciences	89
Dr. C. D. Radford, Manchester, England	76
Research Institute of Natural Resources, Japan	80
South Australian Museum, Australia	96
United States National Museum, Washington, D.C.	1,737 (6)
University of Hokkaido, Japan	78
University of Kansas	90
University of Tokyo, Japan	94
Miscellaneous	800 (7)

Table I. Statistical Summary of Activities Continued

Small mammal skins and skulls

Hemorrhagic Fever Research Team	36
37th Preventive Medicine Company	15
6th Preventive Medicine Survey Detachment	12
207th Preventive Medicine Survey Detachment	20
United States National Museum	76

-
- (1) Estimated; collections primarily to replenish colonies.
 - (2) Estimated; some forms identified without slide preparations.
 - (3) For each species numerous separate illustrations were made; see discussion.
 - (4) Represents estimated number of temporary slide mounts to check identity of larvae collected to replenish colonies.
 - (5) All identifications by Alan Stone, Division of Insect Detection and Identification, Bureau of Entomology and Plant Quarantine, U.S. Dept. of Agriculture, Washington 25, D.C.
 - (6) Part actually transmitted in January 1953.
 - (7) Estimated; to Eighth Army and Japan Logistical Command preventive medicine units and individuals engaged in survey activities.

Tokyo and environs, Omiya and adjacent sections of Saitama Prefecture, and Kyoto and environs. In addition to these recurring field activities there were numerous special surveys of regions more remote from the laboratory locations. Initially, because of the importance of gaining information on rodent and rodent ectoparasites, those studies received primary attention. Later, an increasing amount of effort was directed toward other groups, particularly black flies (Simuliidae). No effort was made to accomplish intensive mosquito surveys, and with the exception of ecological studies on Culex tritaeniorhynchus, observations on Culicidae were more or less incidental to other activities. The areas and times at which special surveys were conducted are shown in Table II. The more important developments from these surveys are summarized in the discussion which follows.

Surveys for Ectoparasites of Small Animals - The accumulated data resulting from this activity are far too extensive to tabulate in this report, and at the same time too meager to assure accurate deductions concerning limits of geographic and seasonal distribution or the range of host specificity. The discussions here presented are preliminary in nature and intended largely as a guide to future survey activities.

Various methods of collecting ectoparasites were used during the course of these surveys, although the basic approach was to capture the host and examine it. For the most part, removal of parasites from the host was by visual examination of the body, augmented by brushing or combing. A modification of the washing technic described by Lipovsky ⁽¹⁾ for removal of chiggers from their host was used occasionally but not routinely. Within the geographic limits of the survey, the more abundant small rodents and insectivores were trapped in virtually all the common ecological habitats. As a group, birds were poorly sampled. Nests of common birds such as sparrows and swallows were examined for parasites. Bat caves were explored on numerous occasions, and captured bats examined for parasites.

Table II. Special Surveys

<u>Locality</u>	<u>Time</u>
Hokkaido	
Hokkaido Pref., Sapporo (Camp Crawford) (Camp Chitose)	April, August
Jozankei	August
Honshu	
Miyagi Pref., Sendai (Ojojihara Maneuver Area)	April, July
Yamagata Pref., Tateoka (Camp Youngmans)	July
Tochigi Pref., Chuzenji	September
Chiba Pref., Kominato	January
Yamanashi Pref., Kofu	January, February, August
Shizuoka Pref., Gotemba (Camp Fuji)	May, August, Sep- tember, October, December
Nagano Pref., Kamikochi	July
Gifu Pref., Gifu	July
Kyoto Pref., Maizuru	August
Hanase	July
Kyushu	
Fukuoka Pref., Hakata (Camp Hakata)	February
Oita Pref., Beppu (Camp Chickamagua)	June
Kumamoto Pref., Kumamoto (Camp Wood)	June

Other collecting methods used included processing of soil samples and contents of animal nests through Berless funnels and the use, on one occasion, of laboratory mice as bait for chiggers. The plate sampling technic for detection of chiggers was also used extensively late in the course of the survey. The exact method used was a modification ⁽²⁾ of the black plastic disc technic described by Wharton and Fuller. ⁽³⁾

Analysis of material assembled permits the following generalizations. In the Trombiculidae, but six species representing two genera are known from Hokkaido, 29 species representing four genera are recorded from Honshu, while similar figures for Kyushu and Shikoku are, respectively, eight and two and eleven and two. The Honshu fauna contains representatives of all generic groups recorded from Japan except Doloiisia which is known from Kyushu. Euschöngastia and Neoschöngastia, and Trombicula subgenus Eutrombicula are not yet recorded from either Hokkaido, Kyushu, or Shikoku. Trombicula (Trombiculindus) and Gahrliopia (Walchia) are not recorded from Hokkaido. Gahrliopia is not known from Kyushu, nor are the subgenera Neotrombicula of the genus Trombicula and Walchia of the genus Gahrliopia. Trombicula (Neotrombicula) and Trombicula (Trombiculindus) are not known from Shikoku. The following graphic presentation of distribution of chiggers will facilitate comparison of records from the various islands (Table III). Only described species are shown in this table.

The laelaptid fauna of Japan is not quite as extensive as the trombiculid fauna. Laelaptid mites have not been reported from Shikoku, and only 17 species have been reported from the other three islands. As in the Trombiculidae, the largest number of laelaptid mite species is found on Honshu, 15 species in all. The representative species found on both Kyushu and Honshu are Bdellonyssus bacoti, Eulaelaps stabularis, Haemogamasus japonicus, Laelaps echidninus, Jettmari,

Table III. Island Distribution of Trombiculidae

(Numbers cited indicate number of collection records)

<u>Species</u>	<u>Hokkaido</u>	<u>Honshu</u>	<u>Kyushu</u>	<u>Shikoku</u>
<u>Dolopsis okabei</u>			1	
<u>Euschöngastia ikacensis</u>		13		
<u>Euschöngastia miyagawai</u>		7		
<u>Gahrlliepia saduski</u>	1	39		1
<u>Gahrlliepia ogatai</u>		1		
<u>Neoschöngastia carveri</u>		1		
<u>Neoschöngastia monticola</u>		1		
<u>Neoschöngastia paenitens</u>		1		
<u>Neoschöngastia posekanyl</u>		1		
<u>Trombicula anous</u>		1		
<u>Trombicula shiraii</u>		1		
<u>Trombicula wichmanni</u>		1		
<u>Trombicula akamushi</u>		2		
<u>Trombicula fuji</u>		78	5	1
<u>Trombicula hasegawai</u>		1		
<u>Trombicula himizu</u>		3		
<u>Trombicula intermedia</u>	6	21		
<u>Trombicula kitasatoi</u>		32	1	3
<u>Trombicula kuroshio</u>		1	4	1
<u>Trombicula miyairii</u>			2	1
<u>Trombicula miyajimai</u>		10		
<u>Trombicula miyazakii</u>	2	1		1
<u>Trombicula pallida</u>		24	1	3
<u>Trombicula palpalis</u>	5	13		
<u>Trombicula scutellaris</u>		22		
<u>Trombicula tanaka-ryoi</u>		9		1
<u>Trombicula tenjin</u>		1		
<u>Trombicula teramurai</u>		2		
<u>Trombicula tosa</u>				4
<u>Trombicula yasukai</u>				1
<u>Trombicula kochiensis</u>		4	2	1
<u>Trombicula japonica</u>		27		
<u>Trombicula mitamurai</u>		2		
<u>Trombicula nagayoi</u>	1	4		
<u>Trombicula pomeranzevi</u>	4			
<u>Trombicula tamiyai</u>		4		

nutalli, and Haemolaelaps glasgowi. The other eight species which comprise the laelaptid fauna on Honshu are Bdellonyssus sylviarum, Ichoronyssus sp., Haemogamasus sp., H. liponyssoides, H. quadrisetatus, Laelaps kochi, Neochoronyssus carnifex, and one new genus and species. Eulaelaps stabularis, Haemogamasus japonicus, and Laelaps jettmari are common to all three islands. Besides these species, a species of Laelaps, Neochoronyssus isabellinus, and N. carnifex are found on Hokkaido.

Eight species of sucking lice (Anoplura) were taken from the rodents and insectivores studied. Of these only one, Polyplax reclinata, was taken from an insectivore, Crociodura dsi-nezumi. Two species of cosmopolitan distribution, Polyplax spinulosa and Hoplopleura oenomydis, were taken commonly from domestic rats (genus Rattus).

The common wild rodent, Apodemus speciosus, was usually infested by both Polyplax serrata and Hoplopleura akanezumi. The latter is by far the most frequently collected species of sucking louse on wild rodents in Japan. It is extremely close to H. affinis and may not be specifically distinct from that species which was

originally recorded from Apodemus agrarius and Apodemus sylvaticus in Europe and has since been recorded from the latter host in Manchuria and Siberia. Apodemus geisha in Japan was found to be infested with the same two louse species as A. speciosus. H. akanezumai was also taken on Mus molossinus.

Hoplopleura acanthopus was recovered from Clethrionomys rufocanus and Microtus montebelli. M. montebelli also was infested with Polyplax abscisa, which is extremely closely related to P. spinulosa and separable from that species only by the structure of the male genitalia. Hoplopleura longula was taken from the harvest mouse, Micromys minutus.

Considering the paucity of our data, distribution records of common individual species are probably more reliable criteria for the detection of distribution barriers than is the evidence furnished by records of higher taxonomic categories. Elements of the holarctic or palaearctic fauna such as Neochoronyssus isabellinus and Trombicula pomeranzevi occur on Hokkaido but are not found on others of the Japanese islands. Trombicula akamushi, the classical tsutsugamushi, has a relatively restricted distribution in Japan, being confined to river basins of three prefectures on Honshu, namely, Akita, Niigata, and Yamagata, all of which drain into the Sea of Japan. Despite the wide range of hosts of Trombicula scutellaris, this species has not been found on either Hokkaido or Kyushu. Trombicula fuji, by far the commonest species in the southeast portion of central Honshu, and known to occur on both Kyushu and Shikoku, has not been found on Hokkaido. Further, although T. fuji has been taken commonly in the central valley area of Yamagata Prefecture, near Tateoka, it was not found during rather extensive work at Ojojihara, near Sendai in Miyagi Prefecture, lying east of the range of mountains that separates the Yamagata valley from the southeast coast of Honshu.

The surveys thus far conducted indicate that on the basis of the seasonal appearance of the parasitic larval stage, chiggers of the genus Trombicula fall into two general categories: a large "winter" group of species, and a small "summer" group. Within these two general categories the occurrence of larval trombiculids tends to be influenced by weather conditions. Our discussion will be concerned primarily with the former group for which our data are more detailed.

The so-called "summer" group consists of Trombicula akamushi and T. tosa, with T. kuroshio, tanaka-ryoi and an unnamed species probably also to be included since they occur during the fall when the weather is still warm. Trombicula pallida might be considered to belong to either group, since this species is found throughout the year, according to the work of Nagayo et al. (4) These workers show that on voles (Microtus), pallida has two yearly population peaks, one in May, the other in late September and October. These same workers clearly show that palpalis and intermedia were most abundant during colder months. With the exception of Trombicula akamushi all species belonging to the "summer" group are found south of the central Honshu area.

With respect to the "winter" group as a whole, the accumulated data indicates that from about the end of May the larval trombiculid population on rodents declines rapidly until the advent of cooler weather some time during September, when the population increases again. No member of the "winter" group of species in Japan is known to be a vector of human disease, although Trombicula intermedia and T. scutellaris have been shown by various investigators (4,5,6) to harbor rickettsiae. Trombicula pallida has also been shown to be capable of transmitting rickettsiae under laboratory conditions. (6)

In general the Laelaptidae show no such definite seasonal distribution as is observed in the larval trombiculids, but our data are too fragmentary to exclude the possibility that there may be some correlation between seasons and the appearance of certain stages of these mites. Of the commoner species of laelaptid mites, such as Eulaelaps stabularis, Laelaps jettmari, and Haemogamasus japonicus, there appears to be a rather uniform distribution of the various stages through all seasons of the

year, with the total population remaining relatively uniform. However, Bdellonyssus bacoti apparently becomes very scarce during late winter, for an intensive search for this species during March and early April of 1952 failed to produce more than an occasional specimen.

Of the host groups explored, rodents and insectivores support by far the largest mite population in terms of numbers of species. Next in importance are birds, and lastly bats. Reptiles, which are favored and apparently normal hosts for chiggers of the genus Eutrombicula in other parts of the world, have not been shown to harbor any trombiculids in Japan. But two species of mites have been taken from bats: Ichoronyssus sp., and an undescribed Trombicula. Neither of these species is known to occur on hosts other than bats. Birds of various species have been rather routinely infested with chiggers, including two species of Neoschöngastia and four species of Trombicula. Of these, the two species of Neoschöngastia and Trombicula anous and T. shiraii, are known only from birds, while Trombicula scutellaris and T. wichmanni have been recorded from both birds and mammals.

Twenty-nine species of chiggers, representing five genera, are known from rodents in Japan. Of these, nine are known from the so-called domestic rodents, Rattus rattus and R. norvegicus. These nine species, namely fujii, kitasatoi, kochiensis, pallida, palpalis, scutellaris, tamiyai, tosa, and wichmanni, all belong to the genus Trombicula and, with the exception of tamiyai and wichmanni, all are also known from wild rodents. The occurrence of chiggers on domestic rodents is believed to be largely fortuitous, and results from the domestic rodents invading habitats normally occupied by wild rodents. This appears not to be a rare phenomenon.

The same factors that are operative in restricting chiggers, in general, to wild rodents appear to be important in restricting the common cosmopolitan laelaptid mites to the domestic rodents in cities. Bdellonyssus bacoti is not known from wild rodents in Japan, although relatively common on domestic rodents in certain areas. Laelaps echidninus and L. nuttalli, very abundant mites on domestic rodents, are rarely found on wild rodents. L. jettmari, common on wild rodents, is but rarely found on domestic species. Thirteen species of laelaptid mites have been collected from wild rodents and six species from domestic rodents. Haemogamasus sp. from Microtus montebelli, Haemogamasus liponyssoides from Dymecodon pilirostris, and Laelaps sp. from Clethrionomys rufocanus have not been found on any other hosts. More significant are the data concerning an as yet unnamed genus and species of laelaptid mite which has been found only on Urotrichus talpoides in both the Kyoto and Camp Fuji areas.

With regard to the chiggers of wild rodents and insectivores, our data are not sufficiently detailed to indicate whether or not host preference or chance are the factors determining the observed host-parasite relationships. A new Trombicula (Trombiculindus) from the Kyoto area occurs primarily on Urotrichus, indicating a strong host preference. On the other hand, such species as Trombicula scutellaris appear to be adaptable to a wide range of hosts, being known from a total of 19 different host species distributed in the Carnivora, Insectivora, Rodentia, and five orders of Aves. In general, it seems that most of the species of Trombicula will attach to and engorge upon any of the Rodentia available, although some may show decided host preference in certain localities. For example, all records of T. intermedia from Hokkaido are from Clethrionomys, although the species is recorded from other hosts on Honshu. Similarly, at Jozankei, Hokkaido, Trombicula pomeranzevi was found in moderate numbers on Clethrionomys in August but was virtually absent from Apodemus in the same habitat, although the latter host was far more abundant. An undescribed genus and species of laelaptid mite found on Urotrichus may be an example of true host specificity. Obviously much additional study will be required to clarify the host-parasite picture.

The site of attachment of chiggers on the host is frequently definitive. For the most part chiggers are found attached at those anatomical areas on a host where the hair is sparse, such as around the genitalia, axillary region of the forelegs, areolar area of the mammary gland, and around the eyes and ears. Certain species of chiggers appear to have preferential sites for attachment to a host. These

observations are not intended to imply that location on a host is fixed for a chigger species, but the occurrence on certain parts of a rodent host tends to give some clues as to what chigger species are present in a given area trapped. At Sapporo, Hokkaido, Trombicula miyazakii collected from Clethrionomys rufocanus was observed to be primarily on the ear rim, whereas Trombicula pomeranzevi was deep in the ear conch on the same host. At Ohara, Kyoto prefecture, the pale chiggers attached about the eyes of Apodemus were Trombicula intermedia, the red species that was found deep in the ear conch was Trombicula fuji, and another red species that was attached on the rim of the ear was Trombicula pallida. In the Camp Fuji area during September 1952, a few elevated volcano-shaped wheals were observed on the abdomen of Apodemus, and chiggers were attached deeply and engorged. Subsequent identifications proved them to be Trombicula scutellaris, which also shows a tendency to attach in and around the ears.

Camp Fuji Chigger Studies. A series of five surveys were made in the Fuji maneuver area during 1952. These surveys were scheduled to permit seasonal samplings of the arthropod fauna and were conducted on the following dates: 22-24 May, 26-30 August, 22-29 September, 7-11 October, and 15-20 December. The area was chosen for observation for two reasons: first, scrub typhus cases are known to have been contracted in this region by troops in 1948 (7), and second, this was the only readily accessible maneuver area for conducting successive surveys. Although the work covered the same fields of study as the general surveys, it became apparent as the work progressed that the chigger data were of particular interest. Accordingly, increased emphasis was placed on the acquisition of data on the seasonal and host distribution and ecology of chigger mites. The discussion which follows deals with this phase of our studies.

The studies carried out were limited to a relatively small region on the north-east slope of Mt. Fuji at elevations of from approximately 2,000 to 4,000 feet. The northern limit of the surveys was in the vicinity of the Kyu-Kamakura highway near Kagosaka Pass in Yamanashi Prefecture; from this locality to about 300 yards beyond station No. 1 on the Gotembaguchi trail, a number of selected areas were studied. For convenience in the tabulation of data, the various areas investigated were numbered serially. The location of these restricted areas within the general Fuji maneuver area is shown in the accompanying sketch map of the region, Figure 1. For a more detailed map of the region see Army Map Service map AMS L874, 1945, Central Honshu 1:25000, sheets 5853 II SE, 5852 I NE, 5852 I NW and 5853 II SW.

Most of the region surveyed is rather uniformly covered with a thick layer of volcanic rock waste of soy bean size and smaller. The soil is generally shallow and in some sections exceedingly spotty. Deep soil is found only along the margins of some of the larger drainage courses. The predominant vegetation consists of various species of grasses ranging from short to medium height, interspersed with shrubs and occasional mature deciduous and evergreen trees. With the exception of Areas 1 and 9, the individual areas surveyed consist mostly of the marginal areas of a series of streams which traverse the area to the north of Camp Fuji. These streams have cut moderate to deep gullies in the volcanic soil and the narrow stream beds have small deposits of alluvial soil. Marginal vegetation consists of heavy growth of grass and shrubbery with some patches of club moss. Away from the stream margins small to medium size trees, both coniferous and deciduous, are found in greater numbers. In areas 4 and 7 grass predominates and shrubs and trees are widely scattered. These areas are rather level and are not traversed by well-defined water courses. The soil surface litter is comprised of a thin layer of grass and leaves. In area 4 thick patches of moss are occasionally found.

Area 1 is the most heavily forested of the sections surveyed. The forest is rather mature and has apparently been harvested regularly. The forest floor is covered with considerable leaf mold, coniferous needles, lichens, and sparse grass. This area is at about 4,000 feet elevation on the slope of Mt. Fuji and is well drained. A wide volcanic ash slide transects the area.

Area 9 is located outside of the area of volcanic rock waste and the soil is composed largely of clay and gravel. Vegetation consists of deciduous and coniferous

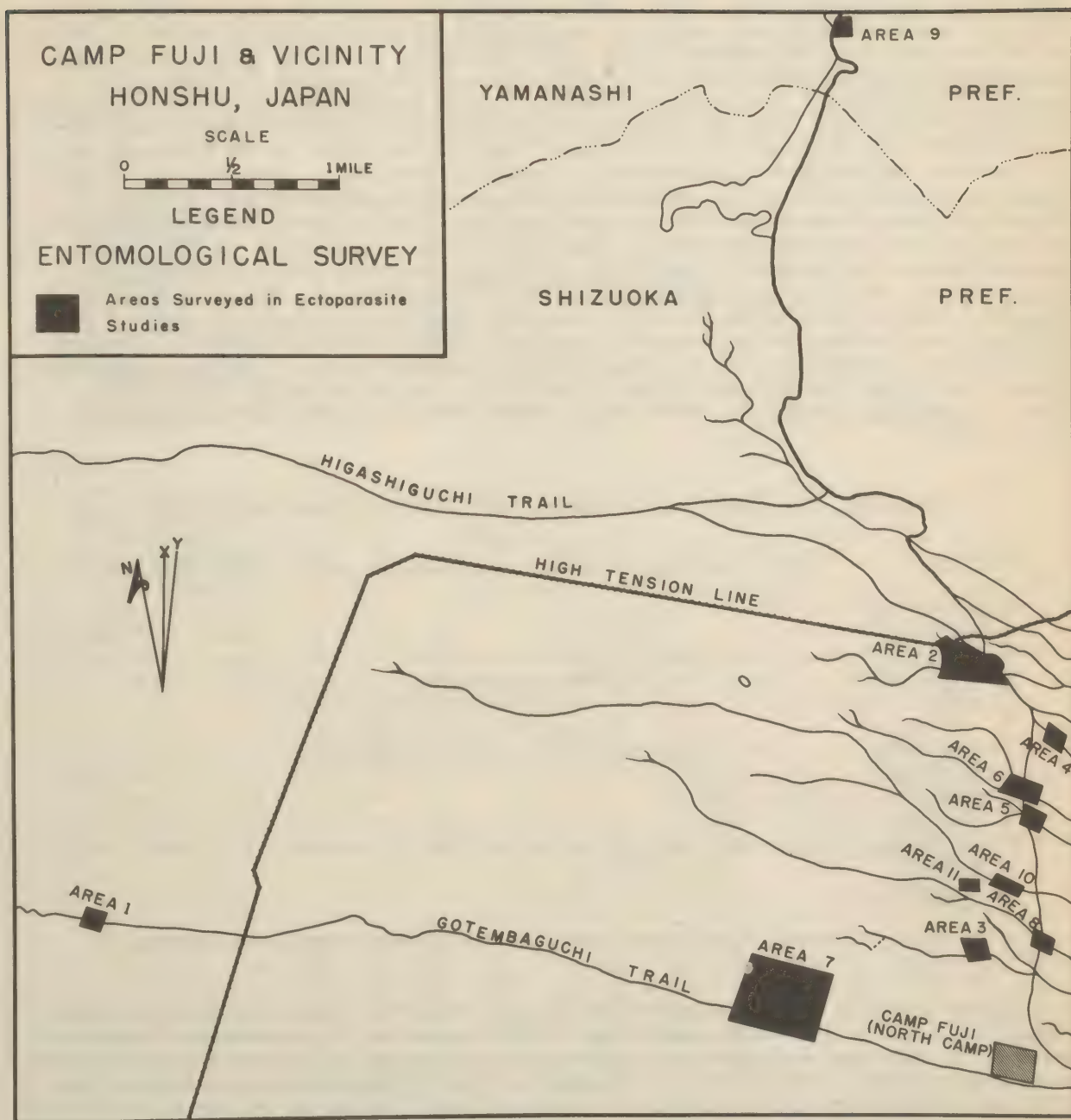


Figure 1. Map of Camp Fuji and Vicinity

trees of moderate height with extensive deciduous undergrowth. The area is well drained and the soil has a thin layer of humus and fallen leaves.

A total of 13 species of chiggers representing four genera were encountered during the Fuji surveys. The distribution of these species by area is indicated in Table IV. These surveys served to support the general observation that larval chiggers were very scarce during the summer, both numerically and in numbers of species encountered. Four species of chiggers were collected in May, two in August, six in September, seven in October, and ten in December (Table VI). In August, scattered specimens of Neoschöngastia posekanyi were taken from birds, and a single specimen of Trombicula nagayoi from a rodent. The autumn increase of larval trombiculids was detected during the September survey. From 22-26 September chiggers were relatively scarce and only 14 specimens were recovered from 57 rodents. Heavy rains began on the night of 25 September and continued intermittently through 26 September. Beginning on 27 September chiggers were relatively abundant in all areas investigated, and many unattached specimens were taken from trapped rodents. Most chiggers found attached to rodents at that time were unengorged, indicating recent attachment. Of the chigger species present in September, Trombicula scutellaris was most abundant, with Trombicula pallida and Trombicula japonica common. No specimens of the common Trombicula fuji were taken at that time, but by October both Trombicula fuji and Trombicula miyajimai were taken. During December Trombicula tamiyai and Euschöngastia miyagawai were added to the growing list of chiggers found in this region. Trombicula fuji was taken in all areas trapped except Area 1, which is at an elevation of about 4,000 feet. T. nagayoi, known from Hokkaido and mountainous areas near Kyoto, was taken only in Area 1.

Table IV. Distribution of Chiggers in Vicinity of Camp Fuji, 1952

Species	Area										
	1	2	3	4	5	6	7	8	9*	10*	11*
<u>E. ikacensis</u>			X				X				
<u>E. miyagawai</u>				X						X	X
<u>G. saduski</u>				X			X		X		
<u>N. posekanyi</u>				from bird, no area							
<u>T. fuji</u>		X	X	X	X	X	X	X	X		
<u>T. japonica</u>	X			X			X	X			
<u>T. kitasatoi</u>								X			
<u>T. miyajimai</u>		X						X			
<u>T. nagayoi</u>	X										
<u>T. pallida</u>							X	X			
<u>T. scutellaris</u>	X	X	X	X	X	X	X				X
<u>T. tamiyai</u>				X							
<u>T. palpalis</u>					X						

* Area 9 trapped only in May; areas 10 and 11 not trapped for rodents

By far the bulk of the chigger material was taken from trapped rodents and insectivores. A total of 312 small mammals and 18 birds were taken and examined for ectoparasites. In addition, chiggers were also collected by means of the plate sampling technique and rodent baiting. The species of chiggers collected from the various hosts and by different methods are shown in Table V, those collected during the different surveys in Table VI. Only those host species found positive at some time during the survey are included in Table V. The small mammals examined for ectoparasites, listed in order of abundance, are shown in Table VII. A total of 18 birds representing seven species were examined, the following three species being negative for chiggers: Emberiza cioides ciopsis, E. spodocephala personata, and Nycticorax nycticorax nycticorax.

Table V. Hosts and Methods of Collection of Chiggers, Camp Fuji Survey, 1952

	<u>E. ikacensis</u>	<u>E. miyagawai</u>	<u>G. saduski</u>	<u>N. posekanyvi</u>	<u>T. fuji</u>	<u>T. japonica</u>	<u>T. kitasatoi</u>	<u>T. miyagawai</u>	<u>T. nagayoi</u>	<u>T. pallida</u>	<u>T. palpalis</u>	<u>T. scutellaris</u>	<u>T. tamiyai</u>
<u>Rodents:</u>													
<u>A. geisha</u>	X				X	X	X	X					
<u>A. speciosus</u>	X		X		X	X		X	X	X	X	X	X
<u>C. smithii</u>						X	X						
<u>M. montebelli</u>	X				X	X				X		X	
<u>M. minutus</u>												X	
<u>Lab. mouse</u>												X	
<u>Insectivore:</u>													
<u>U. talpoides</u>	X		X			X						X	
<u>Birds:*</u>													
<u>C. s. minor</u>				X									
<u>E. f. fucata</u>												X	
<u>S. t. stejnegeri</u>				X									
<u>T. t. fumigatus</u>		X											
<u>Mite Plate:</u>		X			X							X	X

* Chloris sinica minor
Emberiza fucata fucata
Saxicola torquata stejnegeri
Troglodytes troglodytes fumigatus

Table VI. Seasonal Distribution of Chiggers, Camp Fuji Survey, 1952

	<u>E. ikacensis</u>	<u>E. miyagawai</u>	<u>G. saduski</u>	<u>N. posekanyvi</u>	<u>T. fuji</u>	<u>T. japonica</u>	<u>T. kitasatoi</u>	<u>T. miyagawai</u>	<u>T. nagayoi</u>	<u>T. pallida</u>	<u>T. palpalis</u>	<u>T. scutellaris</u>	<u>T. tamiyai</u>
May	X		X		X		X						
August				X					X				
September	X		X			X			X	X		X	
October	X				X	X	X	X		X		X	
December	X	X	X		X	X		X		X	X	X	X

Table VII. Small Mammals Examined for Ectoparasites,
Camp Fuji Survey, 1952

	<u>No. Examined</u>
<u>Rodents:</u>	
<u>Apodemus speciosus</u>	214
<u>Apodemus geisha</u>	22
<u>Clethrionomys smithii</u>	6
<u>Microtus montebelli</u>	5
<u>Glirulus japonicus</u>	2
<u>Micromys minutus</u>	1
<u>Insectivores:</u>	
<u>Urotrichus talpoides</u>	61
<u>Crociodura dsi-nezumi</u>	1

Apodemus speciosus was by far the commonest species of rodent in the region surveyed. Both A. speciosus and A. geisha belong to a genus of woods mice widely distributed throughout Europe and northern Asia. Both species were taken in all ecological habitats trapped; typically they inhabit hilly, marginal agricultural land.

The meadow vole, Microtus montebelli, is a short-tailed mouse found typically in open fields and river flood plains. It has been found to be a reservoir of the scrub typhus rickettsia in endemic areas including those in Niigata Prefecture, Japan. In the Fuji area surveys, Microtus was taken three times in area 7 which is an open grass-covered area, and twice on the margins of the stream in area 5. Of these two areas, the former is the more typical habitat.

Clethrionomys smithii, the red-backed vole, an inhabitant of scrub covered hillsides and forest floor, was taken in areas 3 and 8 which generally fulfill these requirements. This species and the meadow vole are both members of the subfamily Microtinae.

Micromys minutus, the Old World harvest mouse, is a member of the rodent subfamily Murinae, superficially resembling the house mouse in size and coloration. It is typically an inhabitant of grassy flood plains where it builds a nest of grass which is fastened to clumps of grass or weeds 2 to 3 feet above the ground, and resembles nests of certain birds. One specimen of this species was found in area 7.

Although the number of birds taken is too small to permit generalizations concerning host-parasitic relationships, all four of the species found infested frequently feed on the ground.

The animal baiting method and the plate sampling technic were used in an effort to determine the exact micro-environment in which larval chiggers occur. Although the animal baiting method resulted in one positive test, the technic is of limited value because of high mortality of animals, the difficulty of recovery of chiggers, and the time involved in preparation and subsequent inspection of the animals used. The plate sampling technic, although used only during the December survey, appears to have all the advantages of the animal baiting method, and few of the disadvantages.

From a total of 23 "mouse nights" of baiting in areas 3, 4, and 7, specimens of Trombicula scutellaris were obtained with one mouse placed just inside the entrance of a rodent burrow in area 7 on the night of 9 October. The chiggers were attached in the ear and around the anal region. Attempts to isolate rickettsiae from this mouse, from 21 specimens of Apodemus speciosus, and numerous chigger samples from trapped rodents from the Fuji area were all unsuccessful.

Four species of chiggers were found in 9 of approximately 120 plates exposed during the December survey. These plates were used in all areas except number 9, and revealed a very spotty distribution of chiggers even within the limited areas known to produce chiggers by rodent trapping. It was of interest to note that plates placed in or near rodent runs and burrows were not particularly productive as compared with those set on the ground more or less at random. Although some of the plates were exposed for as long as five minutes, exposure beyond two minutes seemed to result in no increase in productivity.

Positive results with chigger plates were obtained in areas 4, 6, 10, and 11. By this method Euschöngastia miyagawai was taken in all four areas, Trombicula fuji and T. tamiyai in Area 4, and T. scutellaris in Area 11. In one instance two species of chiggers (E. miyagawai and T. fuji) were taken from a single plate, and in another instance, in which specimens from two exposed plates were not kept separate, E. miyagawai and T. scutellaris were in all likelihood mixed on a single plate. In all ecological habitats in which chiggers were taken by the plate method, the soil was loose, highly porous volcanic ash with a light covering of grass and leaves or moss and pine needles. All positive plates were exposed between 1000 and 1630 hours, at air temperatures of approximately 40°F.

In our present state of knowledge there appears to be no direct correlation between trapping site and species or number of chiggers taken in the Fuji area. The number of species of chiggers taken from a given area appears to be roughly correlated with the number of rodents trapped and examined. Of the species of chigger encountered, five species, namely Trombicula fuji, T. miyajimai, T. pallida, T. palpalis and T. scutellaris, belong to the subgenus Leptotrombidium, to which all of the known or suspected disease vectors among the Japanese chiggers belong. On the basis of seasonal appearance of the parasitic larval stage, assuming that the 1952 experience is typical (which is by no means necessarily true), T. pallida and T. scutellaris would appear to be the most likely vectors during late September and October, the period during which the 1948 cases must have been contracted. On the same basis, T. fuji, the species found most abundantly in the Camp Fuji area in late October and early November, and T. miyajimai, would appear to be remote possibilities, while T. palpalis, which was not taken until December, would be excluded from consideration.

Correlation of Ectoparasite Surveys of Japan and Korea. Inasmuch as early efforts to solve the problem of the transmission of hemorrhagic fever by laboratory methods were unsuccessful, the investigations turned naturally to a consideration of the epidemiologic approach. Working on the assumption that rodents or other small mammals were the natural reservoir of the disease, and that some rodent ectoparasites were probably responsible for its transmission to man, extensive surveys of rodents and rodent ectoparasites were undertaken by Eighth Army preventive medicine units in Korea. As with all such surveys, the identification of the assembled material proved to be the limiting factor in the analysis of data. The Department of Entomology undertook this task, which required extensive research on the taxonomy of rodent ectoparasites in order to accomplish the identification of several thousand specimens of mites, fleas, and lice. Korean material studied was largely collected between November, 1951 and April, 1952. Although the rodent ectoparasite surveys in Japan were planned as part of a comprehensive study of the medically important arthropods of this region, the importance of information of this type in connection with the hemorrhagic fever problem was immediately apparent. Not only was it desirable to know what potential disease vectors that occurred in Korea were also present in Japan, but a comparison of the two faunas with particular attention to seasonal, host and geographic distribution, and the general ecology and biology of the Japanese species seemed of probable importance in facilitating studies of the same or closely related species in Korea. Following is a preliminary discussion of those phases of our rodent ectoparasite studies that have a rather direct relation to the entomological surveys in Korea.

Eight species of fleas (Siphonaptera), five species of chigger mites (Trombiculidae), and eight species of laelantid mites (Laelantidae) parasitic on rodents

have thus far been shown to be common to both Japan and Korea. No doubt continued work will reveal others. The rodent ectoparasites found in both countries are listed in Table VIII.

Table VIII. Rodent Ectoparasites Common to Japan and Korea

Insects

- Siphonaptera - Xenopsylla cheopis
Pulex irritans
Ctenocephalides canis
Paradoxopsyllus curvispinus
Monopsyllus anisus
Nosopsyllus fasciatus
Leptopsylla segnis
Ctenophthalmus congener

Acarina

- Laelaptidae - Bdellonyssus bacoti
Eulaelaps stabularis
Laelaps echidninus
Laelaps jettmari
Laelaps nuttalli
Haemogamasus japonicus
Haemolaelaps glasgowi
Neochoronyssus carnifex
- Trombiculidae - Euschongastia ikaoensis
Trombicula japonica
Trombicula nagayoi
Trombicula palpalis
Trombicula tamiyai

Several of the above indicated species, such as Xenopsylla cheopis, Pulex irritans, Nosopsyllus fasciatus, and Bdellonyssus bacoti, are important disease vectors of cosmopolitan distribution. Others, such as Ctenocephalides canis, Leptopsylla segnis, Laelaps echidninus and L. nuttalli, have an equally wide distribution but have either not been recognized as vectors of human diseases, or appear to be of little importance in this connection. Most of the other listed species of fleas and laelaptid mites are known to have a wide distribution in the Far East, and the occurrence of some of them in both Japan and Korea would be expected, since the distribution of parasites of domestic rodents is frequently co-extensive with the distribution of the rodents. Prior to the studies accomplished during the past year, there was little information available that would permit comparison of the chigger fauna of Japan with that of Korea.

In Japan Xenopsylla cheopis appears to be exceedingly scarce during the winter months. A search of some 200 domestic rodents (Rattus rattus and R. norvegicus) trapped in Tokyo and urban localities in adjacent Saitama Prefecture during the period February-April 1952, produced no specimens of the oriental rat flea. At no time in our surveys has the species been taken from wild rodents. The dog flea, Ctenocephalides canis is very common on dogs and cats in Japan, and can be found at practically any season. The cat flea, C. felis is exceedingly rare. Comparable data from Korea are not available for these two species.

Monopsyllus anisus, Nosopsyllus fasciatus, and Leptopsylla segnis are common on domestic rodents in Japan and Korea. Ctenophthalmus congener occurs on the two species of domestic rodents and is also found commonly on Apodemus and Clethrionomys on Honshu. On Hokkaido C. congener is represented by a distinct subspecies. Paradoxopsyllus curvispinus is rarely encountered.

Of the laelaptid mites listed in Table VIII, all except Haemogamasus japonicus are recorded from both wild and domestic rodents. H. japonicus is known only from wild rodents and insectivores. Significant features of host and geographic distribution of the Laelaptidae of Japan have been summarized above.

The chigger mites parasitic on rodents appear to be primarily associated with the wild species rather than domestic rodents. This host relationship, in addition to the fact that Trombiculidae are obligatory parasites of vertebrates only in the larval stage, and this for a relatively short period of time, would naturally mitigate against widespread distribution of the species through the agency of man. Unlike the distribution pattern of ectoparasites of domestic rodents, the distribution of chiggers is therefore exceedingly difficult to predict. The available information concerning the listed chigger species is summarized in an attempt to point out significant features.

All the chiggers excepting Trombicula nagayoi appear to belong without question to the "winter species" group. The seasonal status of T. nagayoi is not clear, due to paucity of collection records, but the species has been taken in late August and September in the Mt. Fuji section of central Honshu, and from Hokkaido in September. All species excepting T. tamiyai have been taken from wild rodents in Japan. T. tamiyai was originally described from material taken from domestic rodents from Hachijo island in March, but has also been collected by the plate sampling technic in the Camp Fuji area in December. In the latter locality it is very unlikely that domestic rodents serve as hosts for the species. In Korea T. tamiyai has a fairly wide distribution and has been taken commonly from domestic rodents and occasionally from Apodemus agrarius. It should be noted in this connection that in Korea many of the domestic rodents taken were trapped under field conditions, as is also true of the rodents trapped on Hachijo island.

Geographically, the distribution of these species in Japan is interesting. With the exception of T. palpalis and T. nagayoi which occur from the Yamanaka section of central Honshu north to Hokkaido, all are limited to the southeast side of central Honshu. None is recorded from the southern islands of Japan, or from a region which might imply a tropical distribution. On the contrary, the general distribution of the five species would appear to be in a north temperate region. Of the five species, only T. palpalis belongs to the subgenus Leptotrombidium of the genus Trombicula, which contains the so-called "vector-group" of species. All five have been taken in the Camp Fuji area, where the most striking features of the ecology of the regions studied are the loose, exceedingly porous volcanic ash substratum, thin soil layer with relatively low water-holding capacity, and generally rather sparse vegetative cover. In this area T. japonica was one of the three species the larval stages of which appeared in numbers following late September rains.

Black Fly Studies - Black flies (Diptera: Simuliidae) are of considerable importance as pests in many areas in Japan, although not known to transmit any human disease in this region. However, in other parts of the world certain species of the family act as vectors of pathogenic organisms, and for this reason the group must be considered as of potential medical importance, quite aside from their ability to cause discomfort. Since available information on the ecology and distribution of black flies of Japan (8,9,10) is scanty, it was decided to carry out studies along these lines whenever possible. In addition, black flies or "buyo" were of sufficient abundance and importance in dependent housing areas in Tokyo to indicate need for a special study of that section. During the mid-summer period the Grant Heights dispensary reported that "buyo" bites were responsible for more complaints than any other single affliction.

To most individuals the "bite" of a black fly is at most a mild annoyance; to some it is not even that. But to those unfortunate few who are susceptible to the bites a relatively small number of black flies can be a veritable scourge. In the typical reaction of a susceptible individual, the bite is painless, but is followed

in a short time by the development of a small hemorrhagic spot. Within a few hours a papular lesion develops at the site of the bite, later becoming a vesicular lesion that may persist for days or weeks. Intense itching begins shortly after the bite, and may return periodically, even after the lesions have healed. The itching, and resultant scratching, may cause secondary infection of the lesions. Fortunately, the standard insect repellents are effective in preventing bites, if used conscientiously. Further, although black flies are very wide-spread in Japan, they apparently seldom occur in the enormous numbers that make them unbearable pests in certain regions of northern United States, Canada, and Alaska.

The larval stage of black flies is passed in flowing water. Different species show rather marked differences in preference (or tolerance) of aquatic habitat, some being found only in very swift, cold water, and others occurring commonly in warm, rather sluggish, slightly polluted water courses. Some species are adaptable to a considerable range of conditions; as would be expected, these are the more common and widely distributed forms. Pupae are found in the larval habitat. The mature larva spins a pupal case attached to the larval anchorage, and pupates within the completed case. When ready to emerge the adult floats to the surface of the water enclosed in a bubble and takes flight upon reaching the surface.

Only females seek blood meals, although males may be present in the swarms around a host. The adults are strong fliers, and may be found up to several miles from their larval habitat. Although certain species appear to prefer specific hosts for a blood meal, the common and widely distributed pests of humans will feed on a considerable variety of hosts.

In this survey plants, rocks, and various debris in water courses were searched for the presence of attached larvae and pupae. Whenever possible pupae were placed separately on a piece of moistened paper towel in a small (9 ml) screw cap vial and held for emergence of the adults. The emerged adult, and associated pupa and pupal case were preserved in 70% grain alcohol.

In general, two types of terrain were investigated; the low and relatively flat Kanto plain, inland from Tokyo Bay, with an elevation of approximately 100 feet or less, and the hills of central Honshu at elevations of from 1500 to 3500 feet. In both regions immature stages of black flies were found almost exclusively attached to plants and debris, seldom on rocks, and in greatest abundance on plants growing in shallow water, although the marginal vegetation of deeper streams was also frequently heavily infested. The largest concentrations of immature stages were found in small streams, canals, and roadside ditches.

Streams and small canals in the hilly regions studied were in general cool, clear, and swift, whereas those in the Kanto plain were warm, comparatively slow, and occasionally slightly polluted. In both regions there was a relatively uniform rate of flow in the water courses, and no periodic flushing of the streams by greatly increased heads of water. The different ecological conditions were reflected in the black fly species encountered.

A total of nine species, three as yet unidentified, have been collected and reared during the course of these studies. The most wide-spread species are Simulium venustum and S. salopiense. They are also the most abundant in Tokyo and environs. In the higher elevations other species such as S. latipes, S. tuberosum or one of the unidentified species is likely to be more abundant. S. salopiense and S. venustum conform to the ecological classification of "lowland" species given them by Smart (11) in writing of the British Simuliidae. They are capable of developing in relatively slow-moving and somewhat polluted streams, but immature stages are found most abundantly in shallow water courses with a steady current.

The black fly survey of Tokyo was initiated in mid-September 1952. Since it was not possible to make a complete survey of the city and environs, work was limited to a roughly semi-circular course through outer Tokyo, and to selected areas

within and northwest of the city proper. The geographic extent of the survey, and the areas in which black fly breeding was found, are shown on the associated map, (Figure 2). Only two black fly breeding sites (Areas 11 and 18) were found between "L" Avenue and Tokyo Bay, none between "N" Avenue and the Arakawa drainage. In terms of general topography, black flies were developing only in the higher sections of the city. Streams in the non-producing area were either very slow, very polluted, or lacked vegetation. However, it is probable that earlier in the season black fly breeding occurred over a wide area, for adult flies were known to have been troublesome in the Washington Heights and Ueno Park sections. Of the breeding areas located, the streams to the northwest and southeast of Grant Heights (Areas 1, 2, 3, 16, and 17) had the highest concentrations of immature stages. The typical breeding site in the Tokyo area is a small stream 6 to 8 inches deep, current steady and with a surface speed of about 2 feet per second, stream bed silt, without rocks but often with a growth of Sparganium or similar aquatic vegetation.

The surveys revealed the presence of at least six species in the Tokyo area. During the period September to November venustum and salopiense were the commonest forms and occurred in approximately equal numbers. During this same period three other species were present in small numbers. By late December still another species, as yet unidentified, predominated in the areas under study. It appears that the common summer species, venustum and salopiense, largely disappear with the advent of cold weather and are replaced by one or more "fall" species. Adult black flies were present and annoying on warm days throughout December, but apparently neither venustum nor salopiense was present, although these two species were among those reared from pupae collected on 18 November.

Studies on Biting Midges - In connection with a general survey of biting flies, detailed records were made at Kyoto, Kyoto Prefecture, on the relative abundance of various species of biting midges (Ceratopogonidae) taken in a light trap. A total of nine species, representing three genera, were taken between 2 May and 30 November. Peak populations of the combined species of biting midges occurred during the last half of August. The species represented in the collections, and the proportion of the material belonging to each species, are shown in Table IX.

Table IX. Collections of Biting Midges; Kyoto, 1952

<u>Species</u>	<u>Females</u>	<u>Males</u>	<u>Total</u>	<u>% of Total</u>
<u>Culicoides</u> <u>arakanae</u> (Arakawa)	2412	322	2734	84.64
<u>Culicoides</u> <u>oxystoma</u> Kieffer	129	29	158	4.89
<u>Culicoides</u> sp. new	39	0	39	1.21
<u>Culicoides</u> <u>erairai</u> Kono et Takahasi	35	4	39	1.21
<u>Culicoides</u> <u>sigaensis</u> Tokunaga	20	0	20	0.62
<u>Culicoides</u> <u>kibunensis</u> Tokunaga	16	0	16	0.50
<u>Culicoides</u> sp.	9	0	9	0.29
<u>Forcipomyia</u> <u>metatarsis</u> Tokunaga	149	0	149	4.89
<u>Dasyheila</u> sp.	66	0	66	2.03
	2875	355	3230	

Taxonomic Studies - Problems in the identification of large quantities of rodent ectoparasites from Japan and Korea clearly indicated the need for critical taxonomic studies of certain arthropod groups. Literature dealing with the medical entomology of Korea (12) was particularly deficient. Accordingly, studies of the mites and fleas parasitic on small animals were undertaken. By correlating the taxonomic studies of the Japanese ectoparasites with those on the Korean fauna the value of each study was enhanced. The better known Japanese fauna served to lend perspective to the Korean studies, while at the same time it was possible considerably to extend our knowledge of the potential disease reservoirs and vectors present in each region.

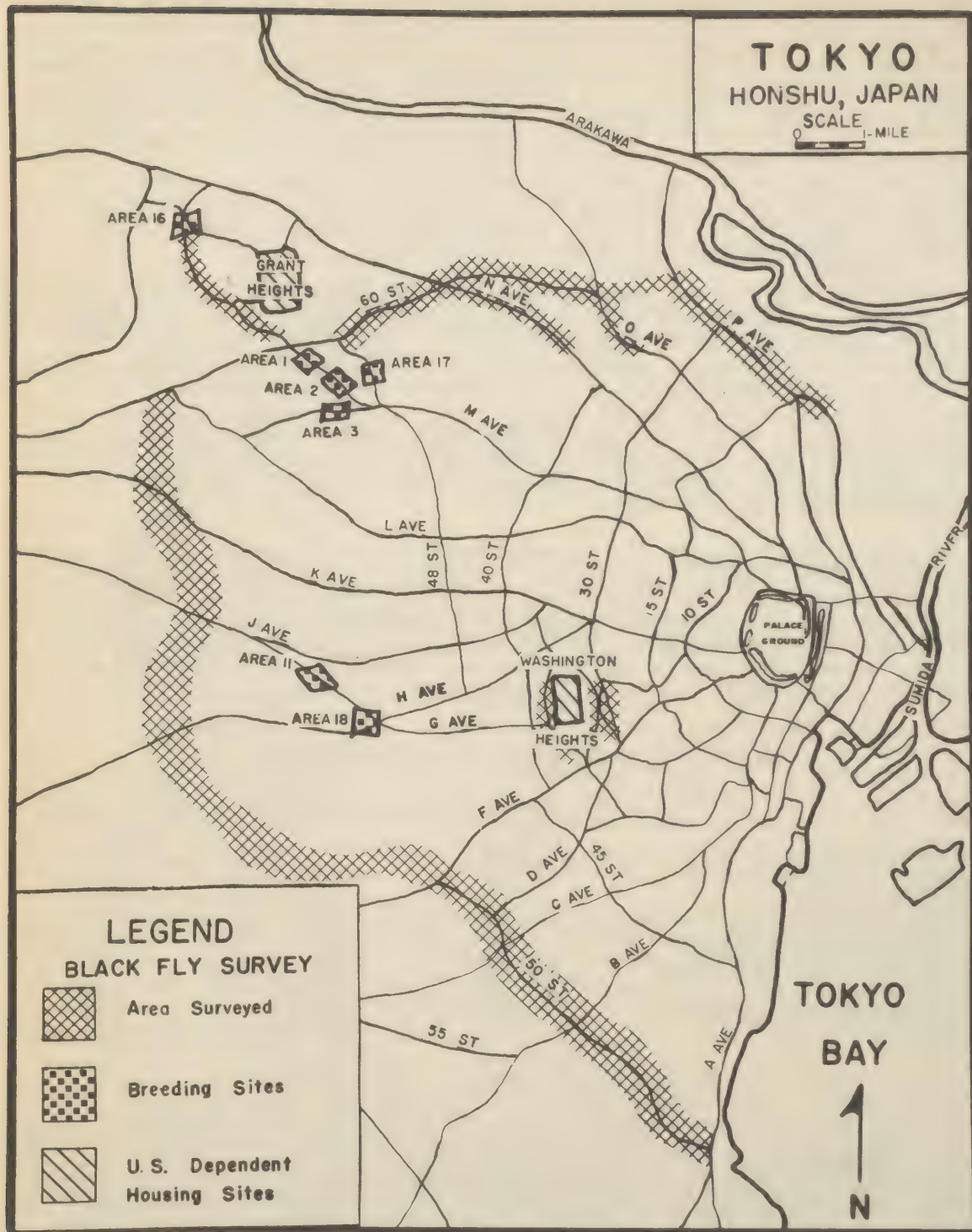


Figure 2. Map of Tokyo

The taxonomic studies undertaken were coordinated closely with those of leading Japanese investigators in the respective fields. Two general types of publications were planned; first, illustrated diagnostic keys and synoptic treatments of the commoner material encountered, and, second, critical basic studies to differentiate all closely related species and forms. The first of these objectives was accomplished for both the mite and flea fauna through preparation and issue of illustrated synopses of these groups in Japan and Korea (13,14). Considerable progress was made toward the second objective. Illustrations used in these technical papers were prepared by a staff of artists of the Taxonomic Entomology Section. Following is a tabulation of illustrations made for the more important of the taxonomic studies.

Siphonaptera (23 species). For each species the following illustrations were made:

Male - head, prothorax, and coxa I; mesothorax and metathorax; sternite and tergite VIII; clasper, finger and sternite IX; enlarged drawing of finger.

Female - terminal segments; outline of sternite VII; anal stylet.

For certain fleas, tarsus, coxa III, and antenna were also drawn.

Trombiculidae (54 species). For each species the following illustrations were made:

Dorsal-ventral views of body; gnathosoma; scutum.

Laelaptidae (12 species). For each species the following illustrations were made:

Male - body; gnathosoma; chelicera.

Female - body, gnathosoma; chelicera; sternal plate; peritremal tube of coxa IV; anal plate.

Same of protonymph and deutonymph, when available.

Identification of Korean Survey Material - At the time the hemorrhagic fever surveys were initiated, our knowledge of the rodent ectoparasite fauna of the Far East was far too fragmentary to permit ready identification of much of the material encountered. Beginning in December 1951, and continuing through March 1952, most of the rodent ectoparasite material assembled by Eighth Army preventive medicine units was referred to this Department for study and identification. This arrangement proved mutually advantageous; it made the material from extensive field studies available for taxonomic study, and, as differentiation of the component species became possible, it made available to the submitting organizations data on the geographic and host distribution of those species. As a direct result of these surveys it was known early in 1952 that the rodent ectoparasite fauna within the then known limits of hemorrhagic fever distribution was qualitatively not different from areas outside the endemic area of the disease, thus indicating the necessity for more refined technic in the epidemiologic approach to the problem. Since all pertinent data were made available to personnel directly engaged in a study of hemorrhagic fever, no detailed analysis of ectoparasite distribution was attempted by this department.

JAPANESE B ENCEPHALITIS INVESTIGATIONS: There exists a wealth of information which suggests strongly that Culex tritaeniorhynchus is the vector of human cases of Japanese B encephalitis (15). The research planned for 1952 was primarily directed toward extending our knowledge of the role of tritaeniorhynchus. The work actually carried out, in part in close cooperation with the Department of Virus and Rickettsial Diseases, embraced three separate but related projects: (1) the biology of Culex tritaeniorhynchus;

(2) JBE experimental infection in mosquitoes; (3) the role of birds in the epidemiology of JBE.

Biology of Culex tritaeniorhynchus - Considerable attention has been directed toward the study of the feeding habits, seasonal abundance, and general ecology of Culex tritaeniorhynchus. Knowledge of factors such as these is not only necessary for a complete picture of its importance as a vector but also valuable in devising means to eliminate or mitigate its attacks. Furthermore, the manipulation of a laboratory adapted colony of this species would presumably be the most expeditious way to study its relationship to the virus of Japanese B encephalitis. Indeed, it is probable that some information of that nature will remain unavailable until such a colony is established. Laboratory adaptation of these mosquitoes might also solve the mystery surrounding their fate in Japan during the winter months, for repeated attempts at finding any stage of tritaeniorhynchus during the colder months have failed.

Studies were begun in the Omiya area of Saitama Prefecture in August 1951 and continued during 1952. The primary effort was directed toward the establishment of a laboratory adapted colony of the species which might serve as a source of stock for virus transmission studies, and for determination of the responses of the mosquitoes to various environmental stresses. These studies were made more difficult by a very low population of tritaeniorhynchus. The collections which were made consisted almost entirely of immature stages and adults which were actually emerging at the time of collection. A statistical approach to the measure of population levels was not attempted due to the limitations imposed on personnel by other duties. Further, previous work appears to have already established, beyond reasonable doubt, that major epidemics of the disease in Japan are associated with an explosive rise in the mosquito population.

Field Observations. Previous workers have noted repeatedly that the initial population of tritaeniorhynchus is confined almost entirely to rice paddies. LaCasse and Yamaguti (16) reported that collections during May through June were confined almost entirely to fresh ground water though there appeared a greater and greater diversity of habitats as the season progressed. Of particular note was the marked increase in rate of incidence in polluted water, particularly in artificial containers. Collections after 10 August 1951, were confined almost entirely to artificial containers, which were largely diluted night soil storage tanks, whereas collections made previous to that time were almost all confined to rice paddies. Although Sasa and Sabin (17) do not discuss the seasonal progression of larval occurrence, they do remark on the fact that tritaeniorhynchus is found in two types of water bodies, one with a low organic content and the other with a relatively high organic content, such as cesspools. Again this season our observations confirmed that the initial population of tritaeniorhynchus larvae was confined almost entirely to rice paddies, irrigation ditches, and small ground pools. This situation persisted until about 15 August, at which time there appeared an increasing tendency for the higher larval populations to occur in breeding sites such as diluted night soil tanks and concrete building foundations. However, larvae were not found in night soil tanks or cisterns until the organic content had become diluted enough to support a growth of surface plants and algae. At no time were tritaeniorhynchus larvae found in such highly polluted tanks as those commonly inhabited by Armigeres subalbatus or Culex pipiens.

The association of immature stages of tritaeniorhynchus with growths of surface plants has been reported previously, (15) and while larvae are not restricted to this type of environment the association is consistent enough to warrant notice. Larvae were rarely collected from locations which were not illuminated by direct sunlight for several hours of the day. Attempts to correlate the larval habitats with pH and water surface temperature were inconclusive. C. tritaeniorhynchus larvae were found in water-bodies whose pH ranged from 6.2 to 7.5, but most abundantly in water approaching the neutral point. Water from night soil containers was on the acid side while that from rice paddies was usually slightly alkaline. The presence or absence of tritaeniorhynchus larvae apparently was not associated with any particular temperature.

Generally the mid-day water temperature of shallow rice paddy pools was five degrees higher than nearby streams or deep pools; however, tritaeniorhynchus was found under both conditions.

Concentrations of larvae were very low in rice paddies, never over 10 per dip, while collections from concrete tanks ranged up to several hundred per dip. This seems to be consistent with the larger water surface of the paddies and the constant dispersion of the population during the process of cultivation. Predators in rice paddies also held the population in check and may account in part for the lack of concentrations of larvae. Paddies or streams which contained numbers of the Japanese Medaka, a minnow, were almost entirely free from mosquito larvae and pupae. In addition, larvae of the beetle family Dytiscidae were frequently seen in active pursuit of larvae. In the diluted night soil tanks tritaeniorhynchus larvae and pupae were frequently taken with the predatory larvae of Culex vorax, and many vorax larvae were observed in the act of eating tritaeniorhynchus larvae. Dytiscid beetle larvae were also present in numbers in some night soil tanks and were frequently seen eating mosquito larvae.

No indication was available in the early season to show how tritaeniorhynchus passed the winter. Collections of adult mosquitoes made during the winter of 1951-52 failed to reveal any hibernating tritaeniorhynchus. By far the largest part of the mosquitoes collected during that time were female Culex pipiens, with C. hayashii and C. orientalis also present in small numbers. An intensive search of caves, dug-outs, buildings, etc. during November and December 1952 likewise failed to produce tritaeniorhynchus. During the same period, a search of water bodies known to support large summer-time populations of tritaeniorhynchus failed to disclose any immature stages. The overwintering habits of this species are therefore still a matter for speculation. Members of the genus Culex typically overwinter as inseminated females, and it seems logical to believe that tritaeniorhynchus should follow this pattern. Opposed to this presumption is the obvious fact that they have not yet been found. The last immature stages which we collected during 1952 were taken near Omiya on 6 October. The adult studies at Shinjima were suspended on 14 September due to the very small number of tritaeniorhynchus being taken. Furthermore, the last adults taken in light traps in the Kyoto area consisted of one female and three males collected on 10 October, although the trap was operated through 30 November.

The mating habits of this species are not known. Mating swarms of mosquitoes noted throughout the season have without exception proved to be pipiens. Neither have the oviposition habits of the species been well studied. The egg rafts are somewhat longer than those of pipiens and each contain approximately 200 eggs which are very similar in appearance to those of pipiens. Eggs which had not yet darkened from contact with the air were collected and hatched in two days at an average temperature of 80°F., so it may be assumed that this approximates the average hatching time.

Laboratory Observations. The greater part of the material brought to the insectary consisted of immature stages, supplemented by a few collections of engorged females from stables in the Tokyo area.

Approximately 60,000 larvae and pupae were collected during 1952. Larvae and pupae were collected in numbers, samples checked for identity, the predators removed, and placed in rearing vessels containing seasoned tap water or water from the collection site. Larvae were fed yeast suspension or ground dog biscuit and thrived on this diet. Throughout the season adult emergence was very successful.

A few egg masses were taken from water bodies which were producing large numbers of immature tritaeniorhynchus. The egg masses were reared, larvae identified and emerging adults kept under the various insectary conditions described below. No difference was detected in the adaptability of adults derived from collected eggs as opposed to those derived from larvae or pupae, or those collected in the adult stage. It had been hoped that mosquitoes reared from the egg stage might prove more adaptable to colonization, but this was not the case.

Adults which were received in the insectary were provided with sugar water and distilled water and after identification were placed in the particular type of cage and environment being evaluated as a holding device. Initially adults were housed in a room size cage with a 13 foot ceiling described in the 1951 report.⁽¹⁵⁾ It was quickly apparent that results would be unsatisfactory as in the past, with adults seldom surviving beyond 48 hours. The cage was kept wet and living plants supplied as resting places. No feeding took place despite a wide variety of hosts offered for varying periods. Artificial lengthening of the daylight hours had no noticeable effect on survival of the adults.

Also used in the culturing attempt were cages one cubic foot in capacity. Adults, together with food and water, were placed in the cages and the cages covered with wet towels to maintain high humidity. In all cases adults failed to feed on hosts offered, no reproduction took place and mortality was very high. Adults were kept at insectary temperature of approximately 80°F, at 85°F in an incubator, and at lower temperature, approximately 50°F, in a refrigerator, but temperature range had very little effect on longevity. Using the same one foot cube cages the effects of variation in humidity were explored. Some cages were kept without covering, others were covered with closely fitted dry cloths and others were covered with wet towels. No differences in longevity of the groups tested appeared significant. In no case did adults reared in the insectary survive beyond 4 days; no mating, feeding or oviposition occurred.

Following these trials cages of cloth and mesh were abandoned for the tritaeniorhynchus tests, and adults were housed in several types of glass vessels. Animal jars $8\frac{1}{2}$ " in diameter and 7" high were covered with a muslin sleeve. Half were left dry and half had the bottom covered with moist cellucotton. Dry and wet jars were placed in the incubator and kept on insectary shelves for comparison of temperature effect. Survival of adults was quite good in the wet jar kept at approximately 85°F in the incubator. Longevity increased to a maximum of 8 days, but feeding and oviposition did not occur.

The method by which maximum longevity was attained made use of glass cylinders $8\frac{1}{2}$ " by 2". The lower end was plugged with moist cellucotton and sealed with a rubber stopper. Up to ten adults were placed in each tube together with a wooden tongue depressor as a resting place and the cylinder top covered with bolting cloth secured with a rubber band. Sugar soaked gauze pads or apple slices were placed on the bolting cloth to provide food. From the beginning males and females in these tubes fed readily on sugar water and some females took blood meals from mice, chickens, and citrated human blood. This feeding is discussed in more detail below. The maximum longevity of adults in these cylinders was 88 days. However, all males died within 21 days and no mating was noted, nor were eggs produced by laboratory reared adults.

Engorged female tritaeniorhynchus collected in horse stables in Tokyo were housed in the glass cylinder cages and produced egg rafts in three cases. These eggs were transferred to seasoned water but either failed to hatch or the hatched larvae did not progress beyond first instar. Lots of pipiens material being reared at the same time and under identical conditions progressed to maturity, so that adverse environmental conditions are presumed not to have been the factors in the non-survival of the tritaeniorhynchus produced in the insectary. Despite the lack of success in establishing a colony of this species, it is felt that the method of caging in glass tubes offers the best hope for future success.

As mentioned above, feeding of blood meals to the adults was not successful except in the case of those caged in glass tubes. In the larger cages the following hosts were offered: black crowned night heron, laboratory rabbit, guinea pig, laboratory rat, adult laboratory mouse, suckling laboratory mouse, adult domestic hen, chick, man, and citrated human blood. The tubed mosquitoes were offered suckling mice, chicks, and citrated human blood and fed readily on all three. When chicks anaesthetized with nembutal were placed on the bolting cloth cover of two tubes the contained female mosquitoes were attracted strongly, probed almost immediately and fed.

In summarizing the data now available and the points still to be explored, certain gaps in our knowledge of the bionomics of this species become evident. First is the question of the mating behavior of the species. Specimens were not observed swarming in nature during this study nor could swarming or mating be induced by crowding in cages in the laboratory. Controlled light conditions or the presence of certain hosts may be necessary to induce mating. Secondly, the question of overwintering is most important and as yet unanswered. If the species overwinters as inseminated females it must do so in very small numbers or in some ecological niche as yet unexplored. In view of the immense numbers of the species encountered in the mid-summer months, overwintering as a small number of females would require a truly remarkable degree of successful reproduction during the spring and early summer months.

Epidemiology of Japanese B Encephalitis - In cooperation with the Department of Virus and Rickettsial Diseases, further investigations on the role of birds in the epidemiology of Japanese B encephalitis were carried out. The specific points investigated were the times at which the virus of Japanese B encephalitis appeared in nestling birds and the mosquito population in nature, whether or not virus free juvenile birds confined in bait traps would show exposure to the virus, and what blood sucking arthropods were attracted to birds so confined. In order to explore these possibilities, bait traps containing juvenile birds were set up at Shinhama bird sanctuary and daily collections and identifications of the trapped insects made. Following identifications of the material, all specimens of blood sucking insects were turned over to the Department of Virus and Rickettsial Diseases for attempted isolation of virus of Japanese B encephalitis. Concurrently isolations were attempted from weekly collections of Culex tritaeniorhynchus made at two resting stations in Tokyo. This was done to give additional assurance that evidence of virus in wild mosquito populations in the general vicinity of Tokyo would not be overlooked and to obtain mosquito material from different habitats attracted to different hosts (horses). Details of the virologic aspects of this work will be found in the report of the Department of Virus and Rickettsial Diseases; the entomological aspects are summarized as follows.

In order to cage the young egrets, herons and chickens to be used as bait, specially designed cage traps were constructed. The cage was three feet square and two feet in height to the eaves, the overall height to the peak of the roof being 2'6". The roof and front were of plywood and access to the interior of the cage was via a 20" diameter circular opening in the front, fitted with a cloth sleeve of the same diameter and approximately 36" long. The cage had a removable metal bottom with a false bottom of 1/4" mesh hardware cloth 4" above the bottom. The removable bottom was to permit easy cleaning of the cage. Sides and rear were of 16 mesh screen, except that the space between the bottom and false bottom was solid construction. A baffle opening to permit entry of mosquitoes extended along the two sides and rear of the cage. The outer baffle opening was 2 3/4", narrowing to 3/4" at the inner opening, the upper face of the baffle being 4" wide and horizontal in position. The baffle was located 15" above the bottom of the cage; 11" above the false bottom.

The bait traps were operated at Shinhama bird sanctuary located on a low alluvial plain at the head of Tokyo Bay about one mile south of the village of Ichikawa. The site of operation was adjacent to the edge of a small pond in the area. The predominant vegetative features of the sanctuary are the dense bamboo thickets growing around the pond and in rather narrow strips out from the pond so as to form a series of "bays" which contain blinds and feeding stations for wild fowl. Aside from the bamboo thickets, which are sufficiently tall to make effective windbreaks, the only large vegetation of consequence consists of scattered pine trees 20-25 feet in height. The sanctuary is occupied during spring and summer by innumerable birds, and thousands of wading birds nest in the dense bamboo thickets. Two of the "bays" formed by these thickets were available for placement of the cage traps. The location of traps within the bays was dictated by the sites at which maximum shading would occur, since it was necessary to prevent excessive temperatures which might result in mortality in the caged birds. The general shape of the bays, and the location of the traps for the greater part of the experiment is shown in Figure 3.

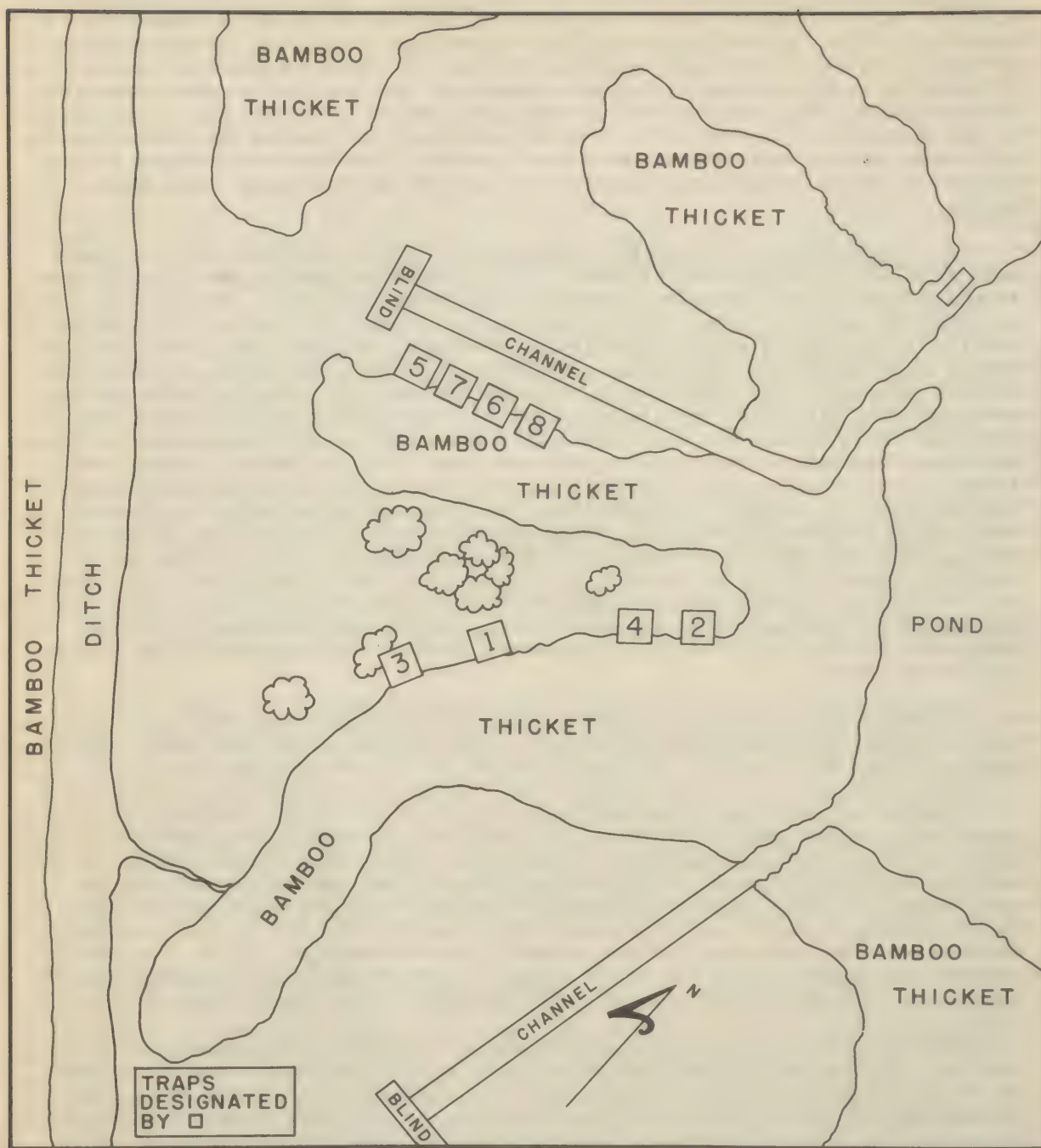


Figure 3. Sketch of a Portion of Shinhama Duck Refuge Showing Location of Mosquito Bait Traps with Relation to Vegetation

During the course of these studies a total of eight cage traps were used, but the exploratory nature of the project led to considerable change as the work progressed. Two cages (nos. 1 and 2), baited respectively with black crowned night herons (BCNH) and little egrets (LE), were placed in a bay close to the edge of the bamboo thicket on 11 July. These two cage traps were in continuous use from that time through the night of 14 September, when the observations were terminated. On 24 July, cages 1 and 2 were exchanged in position. On 25 July these two cages were moved to locations on the ground under the pine trees some 20 feet from the edge of the bamboo thicket. At the same time six additional cages were added, one each baited with BCNH and LE, two baited with chickens and two left empty as controls. These eight traps were paired, and from 25 July through the night of 28 July located under the pine trees in one bay, with one of each pair suspended at an elevation of eight feet above the ground directly above the corresponding cage placed on the ground. On 29 July all cages were located on boxes along the edge of the bamboo thickets as shown in Figure 3, where they remained until the trapping was terminated on 15 September. On 12 August traps numbers 3 and 7, previously used as controls, were baited with BCNH and LE respectively.

All traps were carefully examined each morning and the contained insects captured by means of an aspirator. The living arthropods from each cage were transported to the Tokyo laboratory where they were immediately identified, species and numbers recorded, and specimens of mosquitoes and other blood-sucking insects sent to the Department of Virus and Rickettsial Diseases for attempted isolation of the virus of Japanese B encephalitis. The same procedure was followed in handling mosquito material taken from the Tokyo Police stables and from the Setagaya Race Track stables, except that isolations were attempted from the Culex tritaeniorhynchus specimens only. Results of isolations obtained from these various collections are contained in the report of the Department of Virus and Rickettsial Diseases.

Four species of mosquitoes were taken from the Shinhama bait traps: Culex tritaeniorhynchus Giles, C. pipiens Linnaeus, C. bitaeniorhynchus Giles and Anopheles sinensis Wiedemann. All specimens were females, most of them engorged. The only other blood-sucking arthropods captured were representatives of the genus Culicoides (family Ceratopogonidae), species unknown. C. tritaeniorhynchus was by far the most abundant species of mosquito taken, although pipiens was rather consistently present. The other two species were never present in abundance. Only five specimens of Culicoides were captured.

The small number of traps in operation, and the generally low incidence of tritaeniorhynchus during 1952 (see discussion of ecology of tritaeniorhynchus) make the data available from this experiment too limited to permit positive conclusions or generalizations to be drawn. The following recounting of observed phenomena is primarily for the purpose of focusing attention on points about which further information is needed. Figure 1 in the report of the Department of Virus and Rickettsial Diseases graphically portrays the relative abundance of tritaeniorhynchus caught in the heron and egret baited traps during the course of the work. The seeming marked preference of tritaeniorhynchus for herons over egrets is an item of particular interest, both from the standpoint of the bionomics of the species and its probable significance in connection with the epidemiology of Japanese B encephalitis. The preference of pipiens for herons was less marked, the average number per trap night with herons being approximately two and one-half times that for egrets. No mosquitoes were taken from chicken baited traps, and but three specimens, one tritaeniorhynchus and two pipiens, from the control traps during the time they were operated. The reason for the absence of mosquitoes in the chicken-baited traps has been the subject of considerable speculation, and inquiries designed to clarify this question were not conclusive. It was often observed, during the course of other work, that caged mosquitoes were eaten by chickens placed in the same cage. On the other hand, it seems improbable that chickens could eat every mosquito, if any considerable number of mosquitoes were in the cage. Future plans provide for removal of this element of doubt, both with respect to chicken baited traps and those containing other types of fowl. The possibility, however remote, that this observation indicates a conditioned feeding response by the mosquito population in this area where aquatic fowl abound, has been considered.

Although the period during which traps were elevated eight feet above the ground was very brief, the evidence suggests that the great majority of the mosquito species present in the area and attracted to birds moved at a low level, at least in the open areas of the bays. Whether or not this is the case in the dense bamboo thickets was not determined. It should be noted, in this connection, that air movement in the bays was very slight during most periods.

The number of virus isolates obtained from the trapped mosquitoes, the dates on which virus in the mosquitoes and birds was detected, and the probable significance of these and associated phenomena observed during the work at Shinham are all discussed in the report of the Department of Virus and Rickettsial Diseases. Although rather marked fluctuations were evident in the cage trap mosquito catches, the population sampling method is not considered to have been sufficiently sensitive to permit calling these population "peaks" in the usual sense. On the contrary, evidence from various sources, i.e., Shinham cage trap records, Tokyo resting station records, ecological studies in Saitama Prefecture, and light trap collections in Kyoto and Shiga Prefectures, tends to indicate that there was no sharply defined peak in the relatively low 1952 tritaeniorhynchus population. The Shiga and Kyoto collections indicate high adult populations during the period 24 July-2 August, whereas the data from the Tokyo area indicate that the maximum concentration of adults occurred somewhat later, about 10 August.

From an entomological standpoint these rather limited studies have served to point out new and important avenues of investigation, particularly with respect to species preference among birds acceptable as hosts. Previous work involving wading birds as attractants in cage trap studies (15) did not include segregation of the herons and egrets used, hence the differential attractiveness of the bird species was not detected. The fact that the more attractive (to mosquitoes) of these bird hosts show a higher percentage of "positive" sera in Japanese B encephalitis antibody assays clearly indicates the need for further exploration in this field.

JBE Experimental Infection in Mosquitoes - Data tending to incriminate Culex tritaeniorhynchus mosquitoes in the epidemiology of Japanese B encephalitis have been supplemented by virus isolations from this species (15,18) and by successful virus transmissions.(19,20) These transmission experiments have relied, with few exceptions, on blood-virus suspensions as a source of infection for the mosquitoes. However, the use of virus suspensions as the source of initial infection for the mosquito introduces a highly unnatural factor into transmission experiments, and it was felt that experimental transmission from vertebrate host to vertebrate host should be undertaken. Furthermore, it was believed that experiments to determine the extrinsic incubation period of the virus and explore the possibility of trans-ovarian transmission of the virus were desirable and could be accomplished in conjunction with virus transmission attempts.

For reasons which are discussed in the report of the Department of Virus and Rickettsial Diseases, an avian host was deemed desirable and the chick was chosen as the most logical subject. Use of chicks presented some entomological problems which are discussed below.

When the series of experiments here described were undertaken, Culex tritaeniorhynchus mosquitoes were abundant in nature but not available in a laboratory acclimated colony. It was apparent that wild caught mosquitoes or mosquitoes reared from collected immature forms should not be used because of the possibility of contamination with JBE virus. Therefore, it was decided to use the available colony of Culex pipiens mosquitoes as a source of test animals so that basic information on the behavior of the virus in arthropod tissues could be obtained. Furthermore, manipulation technics developed in the handling of pipiens would be applicable to tritaeniorhynchus should that species become available in the laboratory.

American workers have been unable to isolate JBE virus from pipiens in nature, (15,18) although Japanese workers have reported virus isolations from wild caught

specimens. Culex pipiens has been used repeatedly in successful JBE virus transmission experiments. (19,20,21,22) The species adapts well to laboratory life and breeds readily in a variety of cages. Furthermore, the females feed readily on a wide variety of hosts and are especially attracted to avian hosts. The colony of pipiens used in these experiments was established in July 1951 from material collected near Omiya, Saitama Prefecture, and continued through 1952 with but an occasional addition of new material. The colony was checked on several occasions and found to be free of virus.

Adult mosquitoes were housed in a variety of cages ranging from one cubic foot capacity to room size, but the most satisfactory has proved to be a cage 3' x 3' x 3' with a removable front containing two sleeves and a glass observation panel. Smaller cages present difficulties in the offering of hosts and larger cages make it difficult to control humidity and capture adults. In the cubic yard cage humidity is maintained satisfactorily by covering the cage with saturated turkish towels as described by Trembly. (23)

Females fed on rabbit, guinea pig, mouse, and chicken. More eggs were produced when females were allowed avian blood, but rabbits proved to be the most satisfactory host from point of view of convenience. Auxiliary food for females and males was supplied in the form of dextrose solution on gauze pads and slices of apple. Apple slices were more attractive to the adults but presented a greater problem with mold formation unless renewed daily.

Shallow pans of tap water were available to females for oviposition, and deposited eggs were harvested daily. The rearing vessels used were standard medical issue white enamel basins half full of seasoned water or pond water. Two egg rafts of medium size were placed in each rearing basin which limited the number of first instar larvae to approximately 360-400. At insectary temperature, approximately 80°F., the eggs required about two days for hatching. Eggs could be stored for at least seven days on moist paper at approximately 50°F. and would give satisfactory hatch when warmed to insectary temperature.

The larvae were given a small quantity of yeast suspension on hatching and fed yeast suspension and ground dog biscuit daily as growth progressed. Feeding was a critical item in rearing inasmuch as overfeeding resulted in a surface film which killed larvae, while underfeeding promoted cannibalism and stunting of growth. The rate of larval development was closely correlated with the amount of food, extent of crowding, and especially by the temperature. Larval growth was promoted by temperatures of from 85°F. - 90°F. Pupae were harvested daily and placed in soup bowls for emergence in small cages from which the adults could be transferred either to the main colony cage or held for experimental work.

The attempts to colonize Culex tritaeniorhynchus made use of the same equipment as that described for Culex pipiens. The results of these attempts are discussed in the section devoted to the biology of tritaeniorhynchus. A small colony of Aedes albopictus was maintained in a screened wooden tub during most of the year. This species was not used in virus transmission experiments but served as a source of mosquito eggs for mite feeding.

The size of the blood meal of pipiens, information necessary to calculate the approximate amount of virus obtained by the mosquitoes in taking an infective meal, was determined by weighing a series of 100 each of engorged and unengorged mosquitoes. The average weight of unengorged females was 0.0020 gm. that of engorged females was 0.0039 gm. The average blood meal was therefore approximately 0.002 gm.

The use of chicks as host animals presented a difficulty by handling. An anaesthetic was not used because of its possible effect on the virus. Chicks were confined in small wire cages which restricted their movement; however, the chicks would feed avidly on the mosquitoes if all light was not excluded from the cage during the feeding period. In three test lots of 100 mosquitoes each, three chicks consumed 33, 30, and 12 mosquitoes respectively, when but a small amount of light was admitted to the cages.

In all ten experiments conducted, the female mosquitoes were isolated from recently emerged colony stock in lots of 100 and placed in one cubic foot cages. Water and food, consisting only of apples or sugar water, was withdrawn 24 hours prior to offering the chicks for a blood meal. Initial feeding was considerably better when females were kept with males for two or three days prior to feeding.

Feeding was accomplished by placing the caged chick in the mosquito cages. This procedure, and all others involving possible escape of mosquitoes, was accomplished within a large cage equipped with two sleeves and a glass panel. Immediately after the chicks were placed in the mosquito cages the cages were covered with damp towels and placed on dark warm shelves where they remained for 14-15 hours to assure maximum feeding. The mosquitoes fed lightly during the evening hours but in much greater numbers in the hours between 0600 and 0800. Mosquitoes which refused the initial meal were removed and destroyed and the remaining females supplied with supplementary food and water and kept in darkened cages at temperatures as near the 80° -85°F. range as possible.

A sample of the engorged mosquitoes from each lot were tested for the presence of virus immediately after feeding. At the same time the host chicks were bled and tested for virus. Mosquitoes which engorged on chicks which had not developed viremia were discarded and the remainder pooled. These mosquitoes were used in transmission attempts at 7, 14, and 21 days or were sampled and tested for virus at three day intervals. The protocols for these experiments are discussed in detail in the report of the Department of Virus and Rickettsial Diseases.

All eggs produced by the test mosquitoes were carefully studied in an attempt to detect evidence of transovarian infection. Japanese workers (24) have reported such transmission but our results were negative. In one experiment several egg rafts were obtained from females known to contain virus of a high titer. Half the eggs were ground and injected into mice but no agent was detected. The remaining eggs failed to hatch, although eggs produced at the same time from non-infected females proceeded to develop normally. In one other experiment large numbers of eggs produced by presumably infected females developed normally. It was subsequently determined that the chicks from which these females had received their initial blood meal had failed to develop viremia. In both cases the female mosquitoes had been caged with males prior to feeding for a period sufficient to assure mating.

Throughout the course of the experiments fluctuation of temperature and humidity in the insectary caused considerable loss of both fed and unfed mosquitoes, although engorged females seemed to resist environmental stress far better than unengorged individuals. Of the ten experiments undertaken, six were discontinued due to high mortality in test mosquito lots or failure of the mosquitoes to feed. Japanese workers (25) have reported a strict correlation between temperature and the development of virus in the mosquitoes and lack of proper control of this factor may account for some of the erratic results noted in the virological report.

In these ten experiments, two chicks were infected by the bite of Culex pipiens mosquitoes which had taken an initial blood meal from infected chick and the agent was identified as JBE virus by CF test. In two experiments active proliferation of the virus in the mosquito tissues was demonstrated.

It is believed that the results thus far obtained indicate that this relatively natural method of transference of virus from vertebrate host to vertebrate host has merit and offers a means for the study of the effects of environment on the virus in arthropod tissues.

SUPPORTING ACTIVITIES: In addition to the activities already discussed there were, in the program of the Department, a number of other functions not in themselves research but nevertheless necessary to the investigational program of this or associated organizations. It seems appropriate to class these as supporting activities.

Mammals and Arthropods for Laboratory Experimentation - In the search for laboratory animals in which the etiologic agent of hemorrhagic fever could be demonstrated, it became desirable to experiment with other than the standard laboratory animals. The animals of choice for this line of investigation included wild Japanese rodents and various kinds of arthropods. The Department of Entomology participated in this work by obtaining supplies of wild rodents, and by establishing and/or maintaining numerous arthropod colonies. This brief account of the work covers all arthropod culturing undertaken except that with mosquitoes.

Efforts to obtain an adequate number of the Japanese meadow vole (Microtus montebelli) through local commercial collectors having failed, members of this Department undertook to obtain these animals in sufficient numbers to meet the demand for laboratory animals, and to permit breeding and rearing in captivity. Numerous specimens were trapped alive in the Akabane area of northwest Tokyo. Most of them were used in the hemorrhagic fever experiments, a few retained for attempts at breeding. Four specimens of this species have been reared from young born in captivity, but as yet a perpetuating colony has not been established. In addition to Microtus, a small number of Apodemus speciosus were used in laboratory tests. However, this species does not survive well in captivity, and no young were successfully reared although several were born in captivity.

By far the greater amount of time and effort in this project was devoted to culturing arthropods. A total of eight species of arthropods were maintained in colonies as follows:

Acarina

Laelaptidae - Bdellonyssus bacoti
Laelaps echidninus

Argasidae - Ornithodoros nicolleti
Ornithodoros tholozani

Insecta

Coleoptera - Tenebrio molitor
Tribolium sp.

Siphonaptera - Ctenocephalides canis
Xenopsylla cheopis

The colony of the tropical rat mite, Bdellonyssus bacoti, was established from material collected locally from domestic rats in March 1952, and augmented by specimens supplied from the Army Medical Service Graduate School. Two methods of rearing were used with success. One was essentially as described by Scott et al (26) in which the mites were confined in a container with a half-grown white laboratory rat for a host. The containers used were metal box cages with a moat to prevent the escape of the mites. The cages were filled to a depth of two inches with wood shavings and the mites placed either on the host or in the shavings. New cultures were started periodically as the cage litter became foul and wet.

The second method of rearing bacoti consisted of keeping the mites in glass tubes 2 inches in diameter and 9 inches long, closed at one end by a rubber stopper. Wood shavings were placed in the tube to a depth of 2 inches above the stopper, and held in place by means of a gauze plug. The remaining tube opening was stoppered by a cork covered with fine silk cloth; too tight closure of the tube would result in formation of a vapor film in which mites became entrapped and died. A ring of petrolatum on the outer surface of the tube just below the rim served to trap any mites that escaped when the tube was opened. Mites in the tubes normally sought harborage in the shavings and plug. Blood meals were supplied by placing a hairless suckling mouse in the tube every third day. Mites quickly found the mouse and fed avidly; in

populous colonies the mouse would become covered with all stages of mites and death from exsanguination was fairly common. Mice used for blood meals were normally left in the tubes over night, and after such a period most mites had deserted the host and returned to their harborage. The few remaining mites could easily be dislodged with a fine brush. Tubes containing mite colonies were kept in an incubator at 28°C and approximately 80% relative humidity.

The Laelaps echidninus colony was established from material obtained during the examination of large numbers of domestic rats from the vicinity of Omiya, Saitama Prefecture. Material removed from rats was examined under the stereoscopic microscope and selected adult specimens placed on ectoparasite-free white laboratory rats confined in cages of the same type as was used for bacoti rearing. For this type of mite rearing white laboratory rats have proved quite satisfactory as hosts, since they can be maintained indefinitely without water bottles by supplying them with water-soaked vegetables. Two rearing chambers were established in April 1952 and have been maintained continuously with only an occasional partial change of the shavings used for mite harborage and litter. The chief difficulty encountered in rearing resulted from an occasional rapid increase of Tyroglyphus mites if uneaten rat-food was permitted to accumulate.

The colonies of the two species of soft ticks (Ornithodoros) were established from material originating at the United States Public Health Service, Rocky Mountain Laboratory at Hamilton, Montana. They have been maintained since April, 1952 in 25 x 150 mm. test tubes. The tubes were filled to a depth of 1 to 2 inches with absorbent cotton and plugged with cotton. Strips of paper toweling were placed in the tubes to serve as hiding places and to absorb waste. All the tubes were placed in an incubator at 29°C. and a relative humidity of approximately 80%.

Suckling mice were offered to the ticks twice weekly for a period of 24 hours. Mouse survival was good except for some deaths caused by exsanguination. Molting of the nymphal stages normally followed each engorgement, and death ensued if molting was not accomplished. The feeding interval was irregular, some engorging at intervals as short as eight days while others went for two months between blood meals. Ticks which had undergone long periods of starvation did not engorge at one feeding but required two or three feedings for full engorgement.

During the course of the rearing of these ticks it was possible to make some observations on the biology of O. tholozani. Adult ticks were paired for mating on 19 August and one female which engorged fully on 20 August deposited 78 eggs on 4 October. In the period 4 through 20 October oviposition continued until a total of 135 eggs were deposited. The eggs are spherical in shape, a polished mahogany brown in color, and 0.5 mm. to 0.6 mm. in diameter. They appear to be coated with an adhesive material. As development proceeded the uniformly colored egg became clear at the vegetable pole. High mortality in several groups of eggs apparently resulted from low humidity. The larvae are 0.2 - 0.4 mm. long and 0.25 - 0.35 mm. wide. They are pale brown in color and with dermal ornamentation like that of the mites of the genus Trombicula, rather than with dermal papillae as in the nymphal and adult ticks. When placed on hairless baby mice only 2 of 20 mites were observed to feed. However, feeding was highly successful when the larvae were fed citrated human blood. The blood was placed on cotton in a Stender dish and warmed on a hot plate to approximately body temperature. Larvae were placed on filter paper covering the cotton and engorged rapidly. After feeding, the ticks were placed on filter paper moistened with warm distilled water to remove the clotted blood. Some larvae fed again within four days and to date two have moulted to the first nymphal instar.

The two species of beetles were reared with a minimum of attention. A large stock of meal worms, Tenebrio molitor, was obtained in April 1952, from the Army Medical Service Graduate School. Food at the time of receipt was pulverized bran, but a mixture of oatmeal, corn flakes, and corn meal was substituted because of greater availability. Glass animal jars were used as recommended by Hein (27) and

the food material, interspersed with layers of burlap, filled the lower third. Development of specimens has been rapid and continuous. If the cultures become overcrowded cannibalism results, so the jars were examined once a week and new cultures established if indicated. Mortality is decreased considerably if small amounts of meat are supplied at intervals. Moistened carrot slices, or cabbage leaves, supply moisture and additional food for the beetles. These should be placed on the surface of the rearing medium and renewed every second day. When desired, larvae, pupae or adults may be removed by sieving.

A large number of *Tribolium* beetles were found in a shipment of laboratory animal food in June and transferred to jars containing layers of burlap and ground animal food. Increase in the colony was very rapid and large numbers of all stages are present at all times.

Specimens for the establishment of a colony of the dog flea, *Ctenocephalides canis*, were obtained from dogs in the vicinity of Omiya, Saitama Prefecture, in March 1952, after a thorough search for the preferred *C. felis* failed to produce that species. Fleas were recovered from the dogs with forceps and comb, collection being somewhat easier if the dogs were first dusted lightly with a 2% dust of pyrethrum. When this was done fleas were washed with distilled water before being placed in the colony. Dogs to serve as hosts were dusted thoroughly with 10% DDT powder, washed thoroughly, and placed in an enclosed run. Identified fleas were placed on these dogs and reared according to methods developed by the United States Department of Agriculture laboratory in Orlando, Florida (28). Production of adults has been held at a low but fairly constant rate for this species since the colony was established in March.

Attempts to find the Oriental rat flea (*Xenopsylla cheopis*) in the vicinity of Tokyo early in 1952 having been unsuccessful, specimens of this species were obtained through the Army Medical Service Graduate School. This stock was received in April 1952, and a colony has been maintained since that date. Initially the rearing method used was that developed at the United States Department of Agriculture laboratories at Orlando, Florida (29). However, high humidity produced excessive rusting of the rearing containers and the host rats caused excessive fouling of the rearing substrate, so in September a change was made to the rearing method recommended by Hollenbeck (30). This has proven to be a more satisfactory method in our particular situation. Newly emerged adults feed almost immediately and egg laying begins a few days later. Adults have survived for approximately 6 weeks in rearing chambers without recourse to a blood meal and have produced eggs when allowed to feed.

In the absence of a ready supply of powdered whole blood, a mixture of 50% dry bovine serum albumin and 50% powdered hemoglobin was substituted as food for the larvae of both species of fleas, with excellent results. At 70°F. and 70-80% relative humidity a new generation of fleas appears approximately 5 weeks after seeding of the rearing chamber with eggs.

Preparation of Biological Material. Proper preparation is a necessary prerequisite to the study and identification of biologic material. In order to process the rodent ectoparasites from Japan and Korea approximately 15,000 slide preparations were made and labeled. These slides not only permitted the specimens to be studied and identified, but following identification selected specimens were distributed to units engaged in survey activities in the Far East, and to entomological institutions throughout the world. The collection and identification of ectoparasites, to be effective, must be coupled with positive identification of the mammalian hosts or the avian hosts. Therefore, when this Department undertook an extensive survey of ectoparasites in Japan, as well as an identification service for preventive medicine in the Far East, it became necessary to identify and preserve large numbers of animals. The services of a taxidermist permitted the assembling of a properly prepared and identified collection of rodents and insectivores, from which identified specimens could be supplied to units actively engaged in survey work. Additional specimens of these and other groups were supplied to the United States National Museum for study and confirmation of identification of some species.

Table X shows the number of small mammal skins and skulls prepared during 1952. The number of preparations distributed to various organizations is indicated in the statistical section, Table I. For the most part the preparations were made from animals collected during the course of surveys in Japan, although a few came from Korea and some were supplied by cooperating organizations in Japan.

Table X. Small Mammal Skins and Skulls Prepared, 1952

<u>Species</u>	<u>Number</u>
Rodentia:	
<u>Apodemus agrarius coreae</u>	40
<u>Apodemus geisha geisha</u>	37
<u>Apodemus geisha hokkaido</u>	15
<u>Apodemus speciosus ainu</u>	13
<u>Apodemus speciosus speciosus</u>	142
<u>Clethrionomys rufocanus bedfordiae</u>	37
<u>Clethrionomys rufocanus regulus</u>	4
<u>Clethrionomys smithii smithii</u>	4
<u>Glirulus japonicus</u>	2
<u>Micromys minutus hondonis</u>	4
<u>Micromys minutus japonicus</u>	4
<u>Microtus montebelli montebelli</u>	15
<u>Mus bactrianus yamashinai</u>	5
<u>Mus molossinus molossinus</u>	11
<u>Rattus norvegicus hibernicus</u>	2
<u>Rattus norvegicus norvegicus</u>	6
<u>Rattus rattus alexandrinus</u>	7
Insectivora:	
<u>Crocidura dsi-nezumi chisai</u>	3
<u>Crocidura lasiura thomasi</u>	6
<u>Sorex shinto saveus</u>	3
<u>Sorex unguiculatus</u>	3
<u>Tupai gis</u>	7
<u>Urotrichus talpoides hondonis</u>	20
<u>Urotrichus talpoides talpoides</u>	1
<u>Mogera wogura wogura</u>	6
Chiroptera:	
<u>Eptesicus pallens</u>	1
<u>Myotis macrodactylus</u>	23
<u>Plecotus auritus</u>	1
<u>Rhinolophus ferrum-equinum nippon</u>	2
Carnivora:	
<u>Mustela siberica coreana</u>	1
Total - All Species	425

DEPARTMENT OF CHEMISTRY

The Department of Chemistry consists of six functional sections: Clinical Chemistry, Food Chemistry, Toxicology, Water Analysis, Allergy Investigation, and Miscellaneous Analyses. Collectively these serve as a chemistry laboratory for a variety of analyses with requests from the majority of military organizations within the Far East Command often requiring a variety of procedures and technics. As shown in Table I, the service work load increased in 1952 in comparison with the previous year. Investigational studies are reported under the various sections and are not included in these totals.

Table I. Chemical Examinations

Section	Samples Received			Determinations		
	1951	1952	% Change	1951	1952	% Change
Clinical Chemistry	5,686	7,369	✓ 31.2	9,284	12,940	✓ 39.5
Food Chemistry	2,151	4,012	✓ 86.5	6,948	12,131	✓ 74.6
Toxicology	1,460	1,966	✓ 34.6	9,536	10,679	✓ 13.0
Water Analysis	311	114	- 63.3	2,714	2,217	- 18.3
Allergy Investigation	3,486	861	- 75.3	3,023	1,446	- 52.2
Miscellaneous Analysis	2,095	1,641	- 21.6	5,649	2,703	- 52.1
	15,189	15,963	✓ 4.8	37,154	42,116	✓ 13.3

CLINICAL CHEMISTRY: Services of the Clinical Chemistry Section are available to all medical units of the Far East Command and United Nations Command. The more complex technics of Clinical Chemistry form the bulk of the work since a laboratory of this type acts primarily as a referral laboratory for hospitals and dispensaries which are equipped only for routine procedures. The services of primary standard preparation, procedure evaluations, and consultant assistance are also made available for special studies which may be carried out by hospital units. Personnel and equipment have been assigned to Korea on temporary duty for laboratory studies of hemorrhagic fever and to assist the Surgical Research Team.

Table II lists the types of specimens received and Table III, the procedures performed by the Clinical Chemistry Section during 1952.

Table II. Clinical Specimens Received, 1952

Specimen	No.	Specimen	No.
Blood	2,197	Spinal Fluid	431
Feces	1,161	Urine	513
Plasma	396	Miscellaneous	755*
Serum	1,916	Total	7,369

* Includes calculi, duodenal fluid, lymph, protein-free filtrates, retroperitoneal fluid, saliva, sputum, and synovial fluids.

To meet the requirements of varied medical investigations during 1952, it was necessary to add several new instruments and techniques. Two different types of flame photometers were used. A Baird Associates instrument was obtained first and a Perkin-Elmer flame photometer was acquired shortly thereafter. Before a comparison of the two instruments could be completed, the necessity for observations in

Table III. Clinical Chemistry Determinations

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
A/G Ratio	198	Magnesium, Urine	32
Albumin	198	Methemoglobin	1
Alcohol, Ethyl	936	Nitrogen, Fecal	2
Amylase	27	Non-Protein Nitrogen	46
Ascorbic Acid	2	Occult Blood	1137
Bence-Jones Protein	2	11-Oxycorticosteroids	132
Bile	15	Oxygen Capacity, Blood	1
Bilirubin	166	pH, Blood	3
Bromide	4	Phosphatase, Acid	62
B.S.P.	100	Phosphatase, Alkaline	818
Calcium, serum	210	Phosphorus, Inorganic	107
Calcium, Urine	32	Pipette Calibration	68
Calculi	19	Pipette Delivery Check	624
Carbon Dioxide Capacity ..	7	Pituitrin Concentration ...	1
Cephalin Flocculation	758	Porphyryns, Urine	18
Chloride	89	Potassium	1233
Cholesterol	433	Prothrombin Time	11
Cholesterol Esters	268	Red Blood Cell Fragility ..	412
Creatine	5	Salicylates	7
Creatinine	52	Sodium	387
Fat, Fecal	20	Specific Gravity	2
Globulin (Qualitative) ...	152	Starch, Fecal	1
Globulin (Quantitative) ..	198	Sulfate, Serum, Inorganic .	6
Glucose	536	Sulfhemoglobin	1
Glucose Tolerance	30	Sulfonamides	5
Hematocrit	412	Thymol Turbidity	571
Hemoglobin, Blood	15	Total Protein	633
Hemoglobin, Plasma	767	Trypsin	8
Histamine	177	Urea, Clearance	4
Icteric Index	90	Urea Nitrogen	214
17-Ketosteroids, Urine ...	255	Uric Acid	131
Lead, Urine	4	Urobilinogen	20
Lipase	25		
Magnesium, Serum	40	Total	12,940

hemorrhagic fever patients required the use of a flame photometer in Korea. The Baird was chosen as the more likely instrument for these electrolyte studies and in the early summer it was installed at the 8228th Mobile Army Surgical Hospital. To eliminate direct drafts on the flame a five-gallon coffee can was split and bent to fit the shape of the back of the instrument. A concrete pillar four feet high by two feet square was poured and proved satisfactory for the galvanometer. With internal lithium standards, employed as per the instructions of the manufacturer (1), the Baird Associates flame photometer was operated daily for a period of seven months in the tent laboratory with continuous reproducible results for sodium and potassium. It was demonstrated that the highly sensitive galvanometer, operating apart from the simple flame unit, can be utilized adequately for accurate electrolyte studies under conditions which formerly would have been believed impossible. The easily portable compressor unit also makes this instrument readily adaptable for field use. Heat and humidity during the rainy season created some minor fluctuations of the instrument. These minor difficulties were remedied by insulating the control knobs and working at night.

The Perkin-Elmer flame photometer is much more complex than the Baird Associates instrument. Its use has been restricted to the air-conditioned laboratory where it has proven to be a valuable addition for reducing analysis time for

sodium and potassium. Studies of electrolyte concentrations in various body fluids of subjects undergoing prolonged stress of combat were performed for an investigative team from the Operations Research Office and procedures to expedite examination of multiple specimens of urine and saliva were developed. Experience has shown that the Pyrex type container or the plastic bottle are the only satisfactory containers for collection and preservation of samples for transfer to the base laboratory for analysis and for specimen storage. Soft glass tubes or bottles allow sodium to be dissolved from the glass, contaminating the specimen and producing elevated sodium values.

SODIUM AND POTASSIUM IN URINE AND SALIVA: The available literature is inconsistent regarding electrolyte concentrations in urine and saliva. Widely varying concentrations of sodium and potassium were anticipated, and it was found necessary to experimentally establish normal ranges and average concentrations of these electrolytes. Specimens from a series of control subjects yielded the data in Table IV.

Table IV. Electrolyte Ranges in Urine and Saliva

<u>Specimen</u>	<u>Electrolyte</u>	<u>Range mEq/l</u>	<u>Mean(mEq/l)</u>
Saliva	Sodium	8.7 - 36.5	24.7
Saliva	Potassium	13.5 - 25.0	19.5
Urine	Sodium	24.0 -291.5	140.0
Urine	Potassium	10.9 -195.5	50.0

Such findings render the immediate determination of sodium and potassium in urine and saliva by internal standard methods impossible since any one set of standards will not bracket the wide range encountered. The specimens were initially analyzed by direct intensity flame photometric methods on appropriate dilutions of samples as illustrated in Table V. Suitable concentrations of background elements approximating the averages shown in Table IV were added to the standards.

Table V. Dilution of Saliva and Urine Samples - Direct Intensity

<u>Specimen</u>	<u>Dilution</u>	<u>Standard Range</u>	<u>Background Elements</u>
Saliva	1:25	K - 10-100 ppm Na - 10-100 ppm	23 ppm Na 28 ppm K
Urine	1:50	K - 10-200 ppm Na - 10-200 ppm	64.4 ppm Na 39.0 ppm K

The direct intensity findings were used as a guide for determinations utilizing the internal standard technic for more precise results. The following outline of procedure was found satisfactory. Background elements in concentrations the same as for direct intensity were used. Dilutions of the unknown, based on the approximate values of direct intensity analysis, were made according to Table VI. All dilutions were run against the set of standards indicated in this table. The standards were made up to contain background elements and the same concentration of internal standard as added to the unknown. Appropriate dilution factors must be employed in those cases where the dilution of the unknown sample differs from that used for the standards.

Work with hemorrhagic fever has necessitated the addition of a number of procedures to the routine of Clinical Chemistry. In most cases some adjustment was found necessary in the technics. The more important procedures now employed, as modified by this laboratory, are described in the following paragraphs.

Table VI. Dilution of Saliva and Urine Samples - Internal Standard

<u>Determination</u>	<u>Approximate Concen- tration mEq/l</u>	<u>Sample Dilution</u>	<u>Internal Standard Lithium (ppm)</u>	<u>Standards Concentration & Dilution mEq/l</u>	<u>Standards Range mEq/l</u>
<u>Potassium</u>					
Saliva	10-20	1:50	250	0.2, 0.3, 0.4	10, 15, 20
	20-40	1:100	250	1:50 dilution	
Urine	10-25	1:100	250	0.1, 0.2, 0.3	25, 50, 75
	25-75	1:250	250	1:250 dilution	
	75-150	1:500	250		
	> 150	1:750	250		
<u>Sodium</u>					
Saliva	0-15	1:25	250	0.3, 0.5, 0.7	15, 25, 35
	15-35	1:50	250	1:50 dilution	
	>35	1:100	250		
Urine	25-35	1:25	250	1.0, 1.2, 1.4	125, 150, 175
	35-50	1:35	250	1:125 dilution	
	50-75	1:50	250		
	75-100	1:75	250		
	100-125	1:100	250		
	125-175	1:125	250		
	175-250	1:175	250		
	> 250	1:250	250		

URINARY 11-OXYCORTICOSTEROIDS: The method chosen was that of Daughaday, Jaffe, and Williams (2), which has been modified and adapted to the equipment and requirements of this laboratory. The basis for this method is the reducing power of the urinary corticosteroids. Periodic acid is utilized for the oxidation of urinary extracts with formation of formaldehyde which is then quantitatively measured.

Reagents -

1. Chloroform, CP.
2. Benzene, CP.
3. Sodium Sulfate, anhydrous, CP.
4. Periodic acid reagent: 0.01 M potassium periodate in 0.15 M sulfuric acid (0.23 gm/100 ml).
5. Stannous chloride reagent: 6.0 gm SnCl_2 or 7.14 gm $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$ is dissolved in 10 ml hot conc. HCl , diluted to 100 ml with water and a piece of metallic tin added to stabilize the solution. The crystals of stannous chloride must be colorless and the solution must be discarded when turbid.
6. Chromotropic acid stock solution: 5% chromotropic acid, aqueous. 5.56 gm of reagent grade chromotropic acid (dihydrate) is used for 100 ml. of solution which is filtered immediately and stored in a brown bottle.
7. Chromotropic acid reagent: (Make up fresh for each run). 4.0 ml stock chromotropic acid is diluted with 15.0 M sulfuric acid to 100 ml volume.

8. Alcoholic sulfite solution: 4.0 gm anhydrous sodium sulfite, CP, is dissolved in 100 ml of 1% ethanol.

9. 5.0 M sulfuric acid.

10. 9.0 M sulfuric acid.

11. 15.0 M sulfuric acid.

Preliminary Treatment of Urine Specimens - The homogenous 24-hour urine is shaken with 2-4 gm reagent grade zinc acetate, allowed to stand 10 minutes and filtered. It is desirable for a 16 ounce aliquot of this urinary filtrate to be submitted to the laboratory for analysis for "cortin" and 17-ketosteroid fractions. A minimum of 200 ml of the filtrate is desirable for the Cortin determination, although a smaller amount is used when patients are oliguric and have a 24 hour excretion less than 200 ml.

Procedure -

Extraction: To 200 ml of urine sample, 10.0 ml (5% by volume) concentrated HCl is added and mixed thoroughly. This mixture is then extracted by shaking for 5 minutes with 200 ml chloroform. The emulsion is allowed to break and the chloroform layer collected. Successive extraction with 150 ml and 50 ml portions of chloroform is done and the combined extracts dried for 20 minutes over 40 gm anhydrous sodium sulfate and then filtered. The vessel, sodium sulfate, and filter are washed with 3 successive portions of 15 ml chloroform each. The product of these washings is added to the original extract. The combined extract is evaporated under reduced pressure to a residue in a water bath below 50°C. This is accomplished by drawing calcium chloride dried air through the extract. A two-way stopcock is used to control the rate of flow. Chloroform vapors are condensed in two receptacles which are placed in series in a dry ice-alcohol bath. A cottonseed oil trap is inserted adjacent to the vacuum pump to collect any residual vapors.

The above residue was taken up in 10.0 ml benzene and the flask washed with two 2.5 ml portions of benzene into a small separatory funnel. The benzene fraction was partitioned 3 times with 10.0 ml, 5.0 ml, and 5.0 ml portions of distilled water, centrifuging each time at 800 rpm to break the emulsion. The combined aqueous fraction has 20 ml volume.

Oxidation - A 4 ml aliquot of the aqueous fraction was pipetted into a small distillation flask and 2 ml of the periodic acid reagent added. Oxidation was allowed to proceed for 45 minutes at room temperature after which 1.0 ml of the stannous chloride reagent was added to neutralize excess oxidant. Three ml of 5 M sulfuric acid was added to the flask, giving a total volume of 10 ml. Four ml of distilled water was similarly treated and carried through as a blank.

Distillation - The distillation apparatus used has ground glass joints throughout. Distillation was accomplished by heating with a micro burner until slightly less than half of the solution was carried over. The distillate was collected in a 15 ml graduated centrifuge tube containing 1.0 ml of the sodium sulfite solution. The delivery tip of the condenser must be placed beneath the surface of the sulfite to prevent volatilization loss. Heating must be constant to prevent contents of the receiving tube being sucked back into the condenser. The total volume in the centrifuge tube was adjusted to 6.0 ml by addition of distilled water.

Color Development - 5.0 ml of chromotropic acid reagent were added to 3.0 ml of the distillate in a Coleman cuvette. The tubes (unknown and blank) were heated to 100 C. in a water bath for 30 minutes, allowed to cool, and the final volume adjusted to 10 ml by addition of 2.0 ml 9 M sulfuric acid. The optical density was measured in the Coleman Junior Spectrophotometer adjusted to 100% T at 550 mμ using the blank prepared as above.

Calculations - On a weight basis the amount of formaldehyde formed by the periodic acid oxidation should approximate 8-10% of the amount of steroid. The conversion factor used below represents a 7.6% liberation of formaldehyde. Until isolation and identification of the compounds from the urine made the use of an accurate standard possible, this approximation is regarded (2) as sufficiently accurate.

"Cortin" (or formaldehyde liberating compounds), mg/day = $\frac{10 \text{ DKV}}{200}$

10: Dilution factor of the method.

D: Optical density.

K: Conversion factor of OD to mg Cortin - 0.203. (Determined for instrument of this laboratory using formaldehyde standards).

V: Total volume of the 24 hour urine.

200: Aliquot of urine extracted.

Normals - Limits of normal have not been established with finality but, as employed at this laboratory, the method yields values of 1.0-2.0 mg/day in normal subjects.

Results and Discussion - The reason for pH effect on the yield of urinary cortin remains obscure. It has been suggested that a labile conjugate of the corticosteroids may be susceptible to such mild hydrolysis. Strong hydrolysis, such as used in separation of 17-ketosteroids, results in destruction of the formaldehyde-forming compounds. It was demonstrated (2) that acidification to pH 1.7 before extraction resulted in almost a 2-fold increase in the amount of biologically active corticoids extracted.

The extract need not be washed with water and alkali to remove estrogenic substances and other phenols and pigments since estrogens do not form formaldehyde with periodic acid and urinary pigments do not enter the distillate.

It is believed that the partition with benzene and water increases the specificity of the method since the property of successive solubility in chloroform, benzene, and water is not ascribed to many formaldehyde-liberating compounds.

The possibility of loss during the benzene-water partition presents itself. This was investigated (2) by assaying the benzene and water fractions of a series of urines. Average results of this survey indicate that approximately 90.0% of the formaldehyde-forming compounds were removed in the water phases. This was taken into account in ascribing a theoretical recovery percentage to be utilized in the final calculation.

Due to lack of pure compounds for individual study, the oxidation of the corticoids and similar substances with periodic acid must be deemed to result in variable formation of formaldehyde. This report gives reference to statements concerning the following:

Compounds with ketol side chain yield 1 mole formaldehyde/mole of steroid.

With a dihydroxyacetone type of side chain the reaction forms, under certain conditions, from 1 to 2 moles formaldehyde.

With a glycerol type side chain there is possibility of formation of 2 moles of formaldehyde.

With the method herein used, desoxycorticosterone was found to liberate 1 mole of formaldehyde. This variability of formaldehyde formation indicates that results have a doubtful absolute value but a useful relative value.

The time of oxidation was arbitrarily established at 45 minutes when it was learned there was rapid reaction up to 30 minutes, no appreciable change between 45 and 60 minutes, and only a mild progression thereafter, according to the authors.

SERUM OR PLASMA AMYLASE: The following method is an adaptation of the methods first proposed by Somogyi (3). It varies from the original in that the measure of saccharogenic action of the enzyme is made by estimation of glucose by the Folin-Wu technic whereas Somogyi employed the Shaffer titration method. One-tenth the amount of starch which was proposed is employed. This has permitted freedom from occasional clouding of control filtrates with resultant low amylase values.

Reagents -

1. Starch solution - 150 mg soluble starch, CP, suspended in 5 ml of distilled water is added to 90 ml boiling distilled water. The container is rinsed with an additional 5 ml of water which is added to the boiling solution. The solution is boiled for 1 minute, then incubated in a 40°C. water bath for 15-30 minutes. This solution must be preserved by refrigeration to retard bacterial growth and should be prepared fresh each two weeks.

2. 1% sodium chloride.

3. 5% copper sulfate.

4. 7% sodium tungstate.

Procedure - The test is set up in a series of 16 mm test tubes as follows:

Tube #1 (Unknown) - Place 5 ml starch solution in test tube, warm to 40°C., add 1 ml plasma or serum and mix.

Tube #2 (Starch Control) - Place 5 ml starch solution in test tube, warm to 40°C., add 1 ml distilled water and mix.

To each of tubes #1 and #2, 2 ml of 1% sodium chloride is added and the tubes incubated in a water bath at 40°C. for exactly 30 minutes.

A sample control (tube #3) is prepared while tubes #1 and #2 are being incubated by mixing seven ml. distilled water and one ml. test serum or plasma in a 16 mm test tube. This tube is not incubated.

Immediately after the 30 minutes incubation, 1 ml of 5% copper sulfate followed by 1 ml of 7% sodium tungstate are added to all tubes. Each tube is mixed after addition of each reagent and the solutions are filtered.

Quantitative glucose determinations are run on all filtrates. The Folin-Wu glucose method (4) is used in this laboratory.

Urine Diastase (Amylase) -

The pH of urine must be adjusted to the range of 6.8-7.2.

Acid urines are adjusted with sodium carbonate and alkaline urines are adjusted with acid phosphate.

Phenol red may be used as an indicator.

After urine pH is adjusted, the test is performed exactly as for serum.

Calculation -

$$\begin{array}{rcl} \text{Unknown} & - & (\text{Starch control} \quad \text{Serum control}) \\ (\text{Tube \#1}) & - & (\text{Tube \#2} \quad \text{Tube \#3}) \end{array} = \begin{array}{l} \text{Units of amylase} \\ \text{per 100 ml serum} \\ \text{or urine} \end{array}$$

Interpretation:

Normal: serum 15 - 35 units (i.e. mg glucose liberated per 100 ml serum)

urine 30 - 50 units.

TOXICOLOGY: Toxicological examination was completed on 876 individual cases during 1952. This represents an increase of 41% over the cases completed in 1951. Of the individual cases examined, 215 were autopsies, 618 were clinical cases, and 43 were miscellaneous cases generally examined in cooperation with some other laboratory. The clinical case is the non-fatal case in which body fluids are submitted for examination to determine the presence of suspected toxic substances. The majority of these are individuals who are suspected users of narcotics.

With additional cases and varied problems being presented, a considerable amount of time has been spent on investigation of procedures and evaluation of technics.

Table VII lists the types of specimens received and Table VIII shows the various examinations required to complete work on these specimens.

Table VII. Toxicology Specimens Received

<u>Specimen</u>	<u>No.</u>	<u>Specimen</u>	<u>No.</u>
Ampules	4	Pleural Fluid	3
Blood	647	Spinal Fluid	1
Bone	1	Spleen	1
Brain	152	Sputum	2
Capsules	11	Stomach	1
Cigarettes	22	Stomach Content	158
Crystalline Materials ...	13	Syringe and Needles	15
Feces	2	Tissue, Mixed	7
Gasoline	3	Tooth Paste	3
Gastric Wash	2	Unknown Fluids	20
Heart	2	Unknown Tablets	24
Intestinal Content	18	Urine	489
Intestine	8	Vomit	5
Kidney	157	Water	1
Liver	182	Whiskey	2
Lung	2		
Pancreas	1		
Plant Material	7		
		Total	1,966

Following is a brief review of the more significant positive findings for the year.

Ethyl Alcohol - Sixty-three of the 215 autopsies (29%) examined showed the presence of some ethyl alcohol. This is the same percentage as in 1951.

Carbon Monoxide - Seventeen cases were deaths due to carbon monoxide poisoning. Hemoglobin saturation ranged from 20% to 73.3%. Twelve cases occurred during the first three months of the year.

Table VIII. Toxicology Examinations Completed

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Alcohol, Amyl	9	Marihuana	20
Alcohol, Ethyl	720	Morphine	2,379
Alcohol, Methyl	497	Naphthalene	6
Aldehydes	932	Nicotine	8
Alkaloids, Other	2,504	Opium	4
Arsenic	10	Phenols	635
Barbiturates (Qual.)	1,321	Pine Oil	8
Barbiturates (Quan.)	26	Pyribenzamine	12
Bromides	17	Quinidine	6
Carbonates	9	Quinine	8
Carbon Monoxide	99	Salicylates	18
Chlorides	14	Silica	7
Codeine	150	Starch	6
Cyanide	483	Sulfonamides	12
Halogenated Compounds	486	Miscellaneous	72
Heavy Metals	197		
Hydrocarbons	4	Total	10,679

Chlorides - Comparison of chlorides in left and right heart bloods was made in 5 cases of suspected drowning.

Methyl Alcohol - This substance was detected in only one autopsy this year as compared to 13 cases in 1951.

Diethylene Glycol - One autopsy was encountered in which diethylene glycol was recovered from the stomach contents.

Pine Oil - This substance was found in the stomach content submitted on one attempted suicide case.

Naphthalene - Recovered from the stomach content submitted on one case.

Alkaloids - Alkaloids were recovered from the tissues and fluids submitted on 80 cases during the year. Thirteen of these were autopsy and 67 were clinical. Morphine was the alkaloid most frequently encountered, but codeine was found in 4 cases.

Barbiturates - The specimens submitted on 8 autopsies and 55 clinical cases showed the presence of barbiturates.

Arsenic - This substance was detected in the urine submitted on one case.

Miscellaneous Specimens - Numerous miscellaneous specimens, usually suspected narcotics, were received for examination and identification. The findings on these specimens included heroin, morphine, codeine, crude opium, marihuana, various barbiturates, amphetamine, quinine, quinidine, sulfonamides, foot powders, bromural, and salicylic acid.

Progress on various research projects aimed at more accurate, reliable, and shorter toxicological methods is reported below.

Methyl Alcohol - The problem of detecting small quantities of methyl alcohol, especially in tissue specimens contaminated with formaldehyde, has been solved by the use of a method not dependent on the reduction of methyl alcohol

to formaldehyde and the subsequent detection of the formaldehyde. This is an adaptation of several established methods and was obtained from the Army Medical Service Graduate School. It involves the separation of methanol from tissue or fluids by the use of a special all glass steam distillation apparatus. The alcohol is then separated from the distillate by rectification and a derivative is prepared using 3,5 dinitrobenzoyl chloride (5). The derivative is identified by its melting point. The preparation of the derivative is accomplished on a micro scale using a microsublimation technic. The detailed procedure for distillation and rectification are given in Gradwohl (6). The rectified material is collected in a micro test tube contained in a beaker of crushed ice. A small amount of 3,5 dinitrobenzoyl chloride is added to the alcohol and the tube is gently warmed in a water bath for a few minutes. The contents of the tube are placed in the bulb of a microsublimation apparatus, connected to a vacuum pump and placed in a tube furnace. The tube of the sublimation apparatus is lined with a piece of Visking tubing and the apparatus is placed in the tube furnace in such a manner that this part of the apparatus extends out of the furnace and receives no heat. A thermometer is placed in the furnace, suction is applied, and the temperature is raised to 100°C. where it is maintained for about one hour. If methanol is present, crystals of the 3,5 dinitrobenzoate will form on the Visking tubing lining the sublimation tube and can be removed for a micro melting point determination. The methanol derivative should melt at 107°C. Ethyl alcohol gives a derivative melting at 93°C. The method has been helpful in establishing the presence of very small amounts of methyl and ethyl alcohol in specimens.

Alkaloids - With few exceptions, every toxicological specimen received is routinely examined for the presence of these narcotics. A new method for the extraction of alkaloids from body fluids has been developed in this laboratory and the results gained in hundreds of extractions have shown the method to be superior to any used in the past.

METHOD FOR URINE: Gross and Thompson (7) have shown that acid hydrolysis will permit the extraction of that portion of morphine present in a conjugated form in urine. Fifty to 100 ml are placed in a 500 ml Erlenmeyer flask and subjected to hydrolysis by the addition of concentrated hydrochloric acid, 10% by volume, and autoclaving at 15 pounds pressure for 30 minutes. After cooling the specimen is transferred to a 250 ml centrifuge bottle and 25 to 50 ml of chloroform are added. The bottle is stoppered, shaken for three minutes and centrifuged for about 15 minutes at 3000 rpm in an International size II centrifuge. With the aid of a 50 ml volumetric pipette and rubber bulb as much of the chloroform layer as possible is removed and filtered through filter paper. The extraction is repeated a second time and the chloroform is combined and evaporated in a small beaker. The residue is examined for barbiturates and other acid and neutral substances.

Experience has shown that hydrolysis produces a dark brown discoloration in many of the normal constituents of urine. This material is extractable with chloroform from an acid solution so that the residue in most cases is not entirely suitable for examination. Where barbiturates are suspected, a portion of urine is extracted without hydrolysis and this residue is examined for barbiturates.

The aqueous solution remaining in the centrifuge bottle is made alkaline with 10% sodium hydroxide solution and extraction with chloroform, in two portions, is repeated. The residue will contain alkaloids other than morphine. For the extraction of morphine, the aqueous solution is made acid with hydrochloric acid and then basic with ammonium hydroxide solution. Two extractions are made with a chloroform alcohol 9:1 mixture.

The residues of the two alkaline extractions are next screened and purified by a paper chromatographic method, described in detail elsewhere in this report.

The residues obtained from the extraction of urine after hydrolysis are not suitable for the usual methods of examination involving color reactions and crystalline formation without first applying some form of purification. The use of

various solvents in purification of these residues has been attempted with only limited success. Development of the chromatographic technic has eliminated the need for further investigation on this matter.

METHOD FOR BLOOD: Specimens of blood are extracted in the same manner as urine with the exception that there is no hydrolysis. The blood specimen (20 to 30 ml) is placed in a centrifuge bottle and an equal volume of distilled water is added. The solution is made acid with 3 N hydrochloric acid. Caution must be exercised at this point or separation of the chloroform and aqueous phases will not be possible. It has been found that 8 drops of 3 N hydrochloric acid for 20 ml of blood will result in proper acidification. An excess of acid will result in an emulsion which can only be broken with difficulty. From this point, extractions are carried out as described for urine.

The residues obtained in the extraction of blood are as pure as those obtained by other methods and it is possible to examine them without resorting to further purification, although in this laboratory all extracts are routinely purified by paper chromatography.

Barbiturates - A new method placed in use this year is the Goldbaum (8) ultraviolet procedure for the determination of barbiturates. The original method has been modified following personal communication from Dr. Goldbaum. With this procedure quantitative work is possible and identification can be made in cases where one of the common barbiturates is encountered.

An attempt to develop a satisfactory quantitative method for the determination of barbiturates using the isopropylamine and cobalt chloride reaction (9) was abandoned when it was found that the intensity of the color developed was dependent upon the concentration of both cobalt chloride and the barbiturate.

FOOD CHEMISTRY: The Food Chemistry Section, established late in the year of 1950, functions as a quality control laboratory in the analysis of recombined milk products produced by the several processing plants in Japan, in the analysis of components of the Republic of Korea field ration, and other food products purchased from local contractors. It functions as a nutritional reference laboratory in the analysis of foods for proteins, fats, carbohydrates, fiber, ash, moisture, caloric content, ascorbic acid, thiamine, riboflavin, and calcium.

Space is a limiting factor in the amount of work that can be accomplished. The section moved in April to a separate room which provides more working area. However, with the increase in the number of samples and the multiplicity of tests requested, little or no space is available for investigative work other than developing new procedures of analysis for products submitted from the field.

Methods of analysis follow the procedures of "Standard Methods of Analysis of the Association of Official Agricultural Chemists" (A.O.A.C.), Seventh Edition, 1950, where possible. It has often been found necessary to adopt methods with modifications from other standard tests due to the nature of the sample received when no method is found in "A.O.A.C." Samples analyzed and types of determinations accomplished are shown in Tables IX and X.

The number of samples received during the year varied widely from month to month with a low of 175 samples for January to a high of 439 for February, with a monthly average of 300 compared to 175 for 1951. The number of determinations increased from 7,000 to 12,000.

The analysis of dairy products in the quality control of recombined milk, chocolate milk, ice cream, buttermilk, cream, and eggnog comprised a large part of the work. A summary of the analyses in comparison with 1950 and 1951 appears in Table XI.

Table IX. Food Chemistry Specimens

<u>Sample</u>	<u>No.</u>	<u>Sample</u>	<u>No.</u>
Beef, Ground	2	Dog Food	6
Biscuits, Field, ROKA	113	Fish:	
Cake Mix, Cnd	1	Cod, Dried	2
Candy, Sugar, Korean	1	Cuttle, Dried	31
Cherries, Cnd	30	Sardines, Cnd	2
Chicken, Boiled, Cnd	2	Shrimp, Cnd	3
Dairy Products:		Flour:	
Butter	170	Rice	3
Buttermilk	204	Wheat	11
Cheese, Blue	1	Junket Tablets	35
Cheese, Cheddar	4	Ketchup	1
Cheese, Cheddar, Proc.,		Luncheon Meat	1
Cnd	2	Miso (Bean Mash)	57
Chocolate Milk	690	Orange Drink	1
Cream	81	Pimentos, Cnd	8
Eggnog	7	Ration, Complete, Hosp....	12
Ice Cream	976	Sausage Brine	1
Ice Cream, Mix, Pwd	2	Shoyu (Soy Sauce)	183
Milk, Whole, Raw	1	Tomato Sauce, Cnd	2
Milk, Whole, Past.	3		
Milk, Hom., Past., Recom-			
bined	1193	Total	3842

Table X. Food Chemistry Determinations

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Acidity	211	Levulinic Acid	10
Aldehydes	1	Mercury	1
Antimony	1	Moisture	483
Arsenic	1	Monochloroacetic Acid	1
Ash	60	Nitrates	3
Baume	183	Nitrites	3
Benzoic Acid	7	Nitrogen, Kjeldahl	184
Bismuth	2	Organoleptic	88
Boric Acid	5	Phosphatase, Residual	37
Cadmium	1	pH	1
Caloric Value	12	Protein	76
Carbohydrate	28	Rennin, Quant.	35
Carbon Dioxide	54	Reinsch Test	5
Chlorides, Quant.	106	Saccharin	1
Copper, Quant.	4	Salicylic Acid	7
Curd and Salt	12	Sediment	1139
Fat	3015	Sodium Chloride	180
Fiber	8	Solids not Fat	2935
Fluid Content	2	Starch	3
Formalin	4	Sucrose	10
Glucose	16	Sulfites	1
Glycogen	2	Total Solids	3170
Hydrogen	1	Total Sugar	16
Hexabromide No.....	2		
Iron	4	Total	12,131

Table XI. Examination of Dairy Products

Products	Year	No. of Samples	No. of Samples Low in Butter Fat or Total Solids	Percentage Low
White Milk	1950	53	8	15.1
White Milk	1951	659	106	16.1
White Milk	1952	1193	19	1.6
Chocolate Milk	1950	42	5	11.9
Chocolate Milk	1951	280	14	5.0
Chocolate Milk	1952	690	8	1.2
Ice Cream	1950	32	3	9.3
Ice Cream	1951	580	95	16.4
Ice Cream	1952	976	22	2.3
Cream	1950	None	--	--
Cream	1951	55	15	27.3
Cream	1952	81	2	2.5
Buttermilk*	1952	204	2	1.0
All Dairy Products	1950	137	16	11.7
All Dairy Products	1951	1574	230	14.6
All Dairy Products	1952	3144	53	1.7

* None in 1950, 1951

There has been a major improvement in the quality of the product beginning in January of this year and continuing throughout the year, which coincides in general with the establishment of pecuniary penalties for contractual violations. Prior to this many violations were reported but there was no real incentive for the contractor to improve his product until he became financially liable under the contract.

Fat separation of milk and, to a greater extent, melted ice cream samples was experienced again during the summer months beginning in June and progressively worsening during July and August until the peak was reached in September. With cooler weather making for better shipping conditions, fat separation ceased abruptly. This difficulty was anticipated from our experience in 1951. The separation was not due to souring as only a few samples were soured.

The method of shipping samples to the laboratory was greatly improved about the middle of October, 1952 when insulated wooden containers measuring one cubic foot, lined with sheet metal, and containing two or three sealed, pre-cooled calcium chloride discs as refrigerant were substituted for the inadequate wooden boxes previously used. Prior to use of the new containers samples were received with 0.01, 0.05, and 0.1% formalin added. Some were preserved with a mixture of sodium borate and merthiolate. Others had no preservative added.

The first samples received in the new container were shipped without preservative and arrived in unsound condition. It was suggested that the insulated container be pre-cooled for 24 hours in the ice cream hardening room before shipment. Since that time dairy product samples have arrived in good condition. It is believed that separation during shipment is due to the constant agitation of the product while in transit at temperatures above the melting point of butterfat (28-36°C.). A preliminary study was begun but discontinued due to the press of routine analyses and lack of working space; however, it was discovered that the same type of separation could be produced by shaking the milk in a shaking machine at 300 strokes per minute. Untreated milk

at 20°C. separated in 20 minutes; untreated milk at 30°C. separated in 8 minutes; milk treated with 4 cc of ammonium hydroxide per quart separated after one hour's shaking at 30°C., but separated after 10 minutes shaking when stored at 33°-35°C. for 24 hours prior to shaking.

Early in the year several duplicate samples were exchanged with the International Dairy Supply Laboratory. The samples were analyzed for butterfat and total solids by their technician and by two technicians of this laboratory. Results of analyses between the two technicians of this laboratory agreed well but were not in agreement with results obtained at International Dairy, who were using the Dietert method. This is a short-time, high-temperature (275°C.), forced-air method. Their results were higher for both total solids and butterfat in each instance. It was suggested that they increase the time of drying 10-25% in order that the samples be brought to constant weight. Since that time the Dairy has installed suitable equipment for analyses by the official "A.O.A.C." methods and the two laboratories are usually in close agreement on "companion" line samples.

The residual phosphatase method, as described in "A.O.A.C.", for determining adequacy of pasteurization of dairy products was instituted during the year. One sample of butter from a local Japanese restaurant, as well as samples of butter originating in Denmark, New Zealand, and Australia, were found to give high results. Milk from a local dairy, as well as open market purchases of milk, butter, and ice cream were negative.

Study of the Hynds method (10, 11) for the detection of horse meat in ground meat was continued during 1952 to see if this method could be used as a quantitative, as well as a qualitative method. Hexabromide determinations on the fat of various mixtures of authentic ground horse and beef meat were made and the mixtures tested for glycogen. The results of the analyses are shown in Table XII. The method is rapid, requires no unusual reagents, as does the precipitin test, and can be used for approximate quantitation.

Table XII. Examination of Mixtures of Beef and Horse Meat

<u>Composition of Sample</u>	<u>Glycogen</u>	<u>Mg. Hexabromide/G. Fat</u>
100% Horse Meat	Plus 4	128.0
0% Horse Meat, 100% Beef Meat	Negative	1.6
10% Horse Meat, 90% Beef Meat	Plus 1	28.7
25% Horse Meat, 75% Beef Meat	Plus 2	57.3
50% Horse Meat, 50% Beef Meat	Plus 3	88.2
75% Horse Meat, 25% Beef Meat	Plus 4	111.9

The ascorbic acid procedures were reviewed and revised during the year. The 2,4 dinitrophenylhydrazine method of Roe (12) and the 2,6 dichlorophenol indophenol method of Pepkowitz (13) were investigated. Both methods appear to be quite satisfactory. The indophenol method is the more rapid of the two methods. Roe eliminates diketo-l-gulonic acid (DKA), a biologically inactive oxidation product of ascorbic acid and determines both of the active forms of the vitamin, l-ascorbic acid (ASA) and dehydro-l-ascorbic acid (DHA). Former methods using phenylhydrazine determined not only the active ASA and DHA but also the inactive DKA. The indophenol method is not affected by DKA and was adopted as standard because it is rapid, results are reproducible, the dye need not be standardized, no calibration curve need be constructed, a large amount of sample may be used, and K remains constant for the same photometer and cuvettes. Repeated examinations with the Pepkowitz method showed the K factor to remain relatively constant at varying concentrations of ascorbic acid. The method of Roe followed Beer's law in ascorbic acid concentrations between 5 and 13 gamma.

Methods for the determination of thiamine were investigated. The first method investigated was the colorimetric method of Hochberg, Milnick, and Oser as described by Hawk, Oser, and Summerson (14). The method involves hydrolysis of the sample with 0.05 N sulfuric acid, digestion with takadiastase at pH 4.5, adsorption of the vitamin on a zeolite column, elution of the purified vitamin from the column, and use of the Prebluda and McCallum diazotized amino-acetophenone color reaction to form a red pigment with purified thiamine at pH 9.0. The pigment is extracted with xylene and compared with a blank and standards similarly treated. The comparison, made in a spectrophotometer at wave length 520 mμ, is reasonably reproducible for pure thiamine, omitting the zeolite purification step. However, the addition of copper or iron in the ratio of three parts thiamine to one of heavy metal caused a low result. Chromium did not interfere. Adsorption and elution using Permutit, Folin, after careful preparation with acetic acid and potassium chloride, did not give quantitative or reproducible recoveries. A Japanese product (Vita-Change, Wako-Junyaku Co., Ltd.) gave recoveries no better than Permutit. The Pyke Thiochrome-Ferricyanide Fluorometric modification as described by Winton (15), was investigated. It appears to have merit since it not only eliminates the zeolite column but also because it is simple and directions are given for handling a variety of food products. The method is based on Jansen's observations that thiamine in aqueous alkaline solution, when oxidized by potassium ferricyanide to thiochrome, has an intense blue fluorescent color. The thiochrome is extracted from the aqueous solution with isobutanol and compared in the photofluorometer against a standard thiamine solution with a blank for both sample and standard treated in a manner similar to the sample but containing no ferricyanide. A "Substitute Standard" solution of quinine sulfate is used to set the instrument at 100 on the slide wire potentiometer and zero on the galvanometer. The instrument is checked with the substitute standard before and after each reading and readjusted if necessary. Frequent adjustment is necessary as line voltage fluctuates considerably and no constant voltage transformer is available. Recoveries from 79-104% have been obtained from whole milk, evaporated milk, lettuce, and cabbage using this method.

The fluorometric method for the determination of riboflavin in food, as described in "A.O.A.C." (16), has proven adequate. Analysis using the method was applied to evaporated milk with recovery of 100% of added riboflavin; 98.4% of added vitamin was recovered from wheat flour; 89.6% from fresh cabbage; and 87.0% from fresh lettuce.

Miscellaneous analysis of food has included the determination of moisture and protein in ROKA Field Biscuits; the kind and amount of gas present in cake mix and canned cherries; composition of Korean "Victory" candy for the ROKA Army; amount of iron and copper present in ice cream mix powder; moisture and sand content of rice flour; carbohydrate, protein, fat, ash, and moisture in hospital diets submitted by the Hepatitis Research Team; calcium in canned dog food of Japanese purchase; junket tablets for rennin content; adulteration of ketchup and orange drink in which no adulterants were found; and the nitrate content of a brine from canned sausage.

ALLERGY INVESTIGATION: The main object of the Allergy Investigation Section has been to continue the investigation of the condition known locally as "Yokohama Asthma". Symptoms of this disease and an outline of earlier work have been described previously (17). The continued studies on pollen and fungi spores yielded important, but negative, information. Many aspects of this disease suggest that its cause lies in air pollution similar to that recognized in many industrial cities in the United States (18, 19), where combinations of air pollution, topography, and unusual meteorological conditions cause "smog". In many aspects the symptoms registered by victims of "Yokohama Asthma" are similar to the symptoms of "smog" victims. For the foregoing reasons air pollution in Yokohama was investigated during the latter part of 1952 (Table XIII).

Botanical Studies - The early part of the year was spent on the cultivation of molds and rusts, such as Alternaria, Homodendron, Aspergillus, and Puccinia for preparation of allergenic extracts. The atmospheric slides taken from January to March showed numerous spores of Alternaria and Aspergillus and parts of rice stalks. Studies on algae (seaweed) and fern spores were also made during these months. The drying and packaging of seaweed is one of the major winter industries surrounding Tokyo Bay.

Table XIII. Work Performed by Allergy Section

Material Received for Extraction	21
Plants Collected for Pollen Reference Slides	217
Plates Exposed for Mold Counts	82
Fungi Allergenic Extracts Prepared	19
Inspections made Regarding Yokohama Atmosphere	541
Reported Incidence of Yokohama Asthma	587

During the early spring months from March to June the most significant air-borne pollen found on slides were the cedars in April and pines in early May. These two plants are found abundantly throughout Japan and particularly around Yokohama, where many small stands of cedar forests are located. Pollen of Betula, Ginko, Castanea, Acer, and Alnus were also counted on the slides, but were usually less frequent than the pollen of cedars and pines.

The summer months bring myriads of flowers of various garden types, mostly entomophiles, which have little significance in this study, especially since incidence of "Yokohama Asthma" is very low during this time. The rainy season extending through June and July prevents dissemination of pollens, even those of anemophilous type, such as grasses.

Autumn produces more suspected "hayfever" plants in the Yokohama-Tokyo area than any other season. As in most parts of the United States, the Yokohama area has all of the weeds which are capable of producing hayfever. These include amaranth (pigweed and carelessnessweed), chenopods, atriplexes (saltbushes and Crachs), and various members of the Compositae, such as Ambrosia (ragweeds), Xanthium (cocklebur), Solidago (golden-rods), Artemisia (sagebrush), and Chrysanthemum (cultivated and wild species). These plants begin to flower in mid-August and in the case of Chrysanthemums last through December until the first or second killing frost.

It is probable that many cases of asthma are caused by the pollen of the hayfever producing plants found in this area. However, the greatest incidence of "Yokohama Asthma" occurs during periods when little pollen of any type is produced. Clinical testing of pollen and spores found during the periods of high incidence of "Yokohama Asthma" did not yield any positive results.

Air Pollution Studies - The composition of the atmosphere of Yokohama is not constant. Due to the many heterogenous by-products of industry, the sources of air contaminants are many and varied. Foremost to be considered is the combustion of fuel. Liquid and gaseous fuels discharge sulfur dioxide and all types of organic gases and vapors including methane, acetylene, aldehydes, phenols, ketones, ammonia, and alcohols. The burning of coal discharges soot, water vapor, carbon monoxide, carbon dioxide, oxides of nitrogen, hydrocarbons, and organic acids. Workers in Los Angeles (20) report the following figures of known daily emissions of air contaminants from gasoline combustion alone:

Oxides of Nitrogen	132 tons
Sulfur Dioxide	21 tons
Aldehydes	10 tons
Organic Acids	7 tons
Aerosols	25 tons

Normally all of these products are removed from the air by natural weather processes. However, during certain periods of the year these pollutants may be retained in an area by temperature inversions, weak and alternating winds and high hills and mountains. In Yokohama all these processes come into play during the winter months so that for long periods of time a "smog" settles over the city. It is during these periods that the incidence of "Yokohama Asthma" reaches a peak.

Four problems have been and are still under study. These are:

- (1) Determination of air contaminants.
- (2) Quantities of the contaminants to be found.
- (3) The effect of weather.
- (4) Correlation of air pollution, weather, and incidence of "Yokohama Asthma".

An air-sampling station was established in a laboratory truck behind the Yokohama Army Hospital. Due to limited personnel available for the study, air-sampling could be conducted only from 1900 to 2400 daily. This sampling time had two advantages. It allowed normal duty hours for scarce personnel and it was thought the sampling time would cover a period which would give an indication of the air pollution throughout the day.

The iodine method as outlined by Jacobs (21) was found to be best for detection of small quantities of sulfur dioxide in the air.

The procedure used for the determination of oxides of nitrogen is the method also detailed by Jacobs (21). The absorbing solution consists of 35 ml of 5% KOH solution and 5 ml of 3% H_2O_2 solution. After sampling a known amount of air the solution is analyzed for total nitrate by the phenoldisulfonic acid method.

The method of analysis for ozone utilizes the oxidizing property of ozone on a 2N solution of KI. The free iodine liberated is titrated with standard sodium thiosulfate. This method (22) is specific only in the absence of certain other oxidizing agents which may be eliminated by passing the air to be analyzed through a strong potassium permanganate solution and then a chromic acid solution.

The amount of dust in the air is estimated by a dust index which ranges from 0 to 5. A known volume of air (6.6m) is drawn through two sheets of ether-washed No. 1 Whatman 110 mm filter paper in $2\frac{1}{2}$ hours. The shade of grey-black developing due to impinged dust particles is compared with a standard grey-black color chart prepared with activated charcoal. This method has given highly satisfactory results in evaluation of the amount of dust particles in the air. The amount of ether-soluble aerosols in the air was determined from the same filter paper used to trap the dust. The two sheets were cut into small squares and extracted for 15 to 20 minutes with 25 ml of vacuum distilled absolute ether. The ether was then filtered into a carefully weighed 50 ml beaker, evaporated under vacuum to constant weight, and the weight of the residue determined. This weight represents the amount of ether-soluble aerosol in the air and is a complex mixture of organic acids, ketones, unsaturated hydrocarbons, peroxidic organic compounds and others as yet not fully defined (23).

Rain and wind, unless winds are weak and alternating, are of aid in cleaning the air of contaminants. Important phenomena of weather also contributing to air pollution are temperature inversions. These conditions are produced when the earth and the air next to the ground surface become cooler than the atmosphere above and the thermal gradient becomes inverted. The atmosphere becomes very stable with little or no turbulence and virtually no dilution of industrial effluents (24). Air pollutants are literally trapped next to the ground by a lid of warm air. The effectiveness of a temperature inversion in producing "smog" depends upon the altitude of the lower surface of the warm air, the duration of the inversion, and the presence of air contaminants.

Weather data, including wind direction and velocity, precipitation, and temperature inversion data, for Yokohama and area were furnished by the 2143d Air Weather Wing, USAF. Unfortunately, the data for temperature inversions were collected only twice daily, and then at Haneda Air Station some six miles from Yokohama. Fortunately, the presence of strong low level temperature inversions in Yokohama could be noted by visual observation of the behavior of smoke emitting from stacks (25).

That weather is definitely a factor of importance in a study of asthma has been shown by many other investigations (18, 19, 23, 25, 26, 27, 28). Prolonged periods of little or no rain and gentle winds produce ideal conditions for "smog" development. High hills and mountains exhibit a confining effect during periods when temperature inversions are found at low altitudes. Contaminated air does not escape and the volume of air beneath the inversion is insufficient to dilute the pollutants. Many substances thereby reach objectionable intensities.

"Yokohama Asthma" and Air Pollution - Since the study of air pollution is not yet complete, final results are not available. Certain indications point to air pollution as a major factor in "Yokohama Asthma". With the qualification that data is incomplete the following information is presented concerning the average amounts of contaminants found on days of "smog" formation and on days when little or no "smog" was present:

<u>Contaminant</u>	<u>"Smog"-Free Days</u>	<u>Days of "Smog" Formation</u>
Ether-Soluble Aerosols	0.011 mg/m ³	0.081 mg/m ³
Dust Index	1.0	3.8
Sulfur Dioxide	0.075 micrograms/Liter	0.17 micrograms/Liter
Ozone	0.081 micrograms/Liter	0.17 micrograms/Liter
Nitrogen Oxides	0.26 micrograms/Liter	0.50 micrograms/Liter

The average number of cases of asthma computed for days when air-sampling was possible was 2.4 for "smog"-free days, and 11.4 for days of "smog" formation.

Of the above-listed air contaminants, the ether-soluble aerosols and dust show the closest correlation with incidence rates. (See Figure 1) With the exception of the 29th and 30th of November, 1952 the correlation appears to be excellent. That these days do not show correlation may be due to many factors, several of which are discussed.

It was noted that on four occasions the "smog" persisted for two or more days. During these periods the asthma incidence increased markedly as shown below:

<u>Period</u>	<u>Average Daily Incidence</u>
15-16 November	19 Cases
29-30 November	15 Cases
5-6-7 December	13 Cases
12-13-14-15 December	13 Cases

In each period the average number of daily cases was higher than the general average (11.4) computed for all the days of "smog" formation. At the time of this report weather data were available up to 30 November 1952. On the basis of these data, the following comparison of incidence and weather conditions has been prepared for this period.

	<u>"Smog"-Free Days</u>	<u>Days of "Smog" Formation</u>
Average Wind Velocity	12 m.p.h.	8.3 m.p.h.
Average Rainfall	0.21 inches	0.03 inches
Average Number of Cases*	2.3 patients	12.2 patients

Certain inconsistencies have manifested themselves and further studies are considered necessary to establish the relationship between asthma incidence, temperature inversion data, air contaminants, and other weather factors. It has been noted, however, that there is apparent correlation between high incidence of asthma, high concentrations of air contaminants, and "smog" formation. The formation of "smog" generally conforms to weather conditions of low wind velocities, little or no rain, and low-level temperature inversions.

Certain problems have arisen in this study. They are concerned primarily with case incidence and personnel shortages.

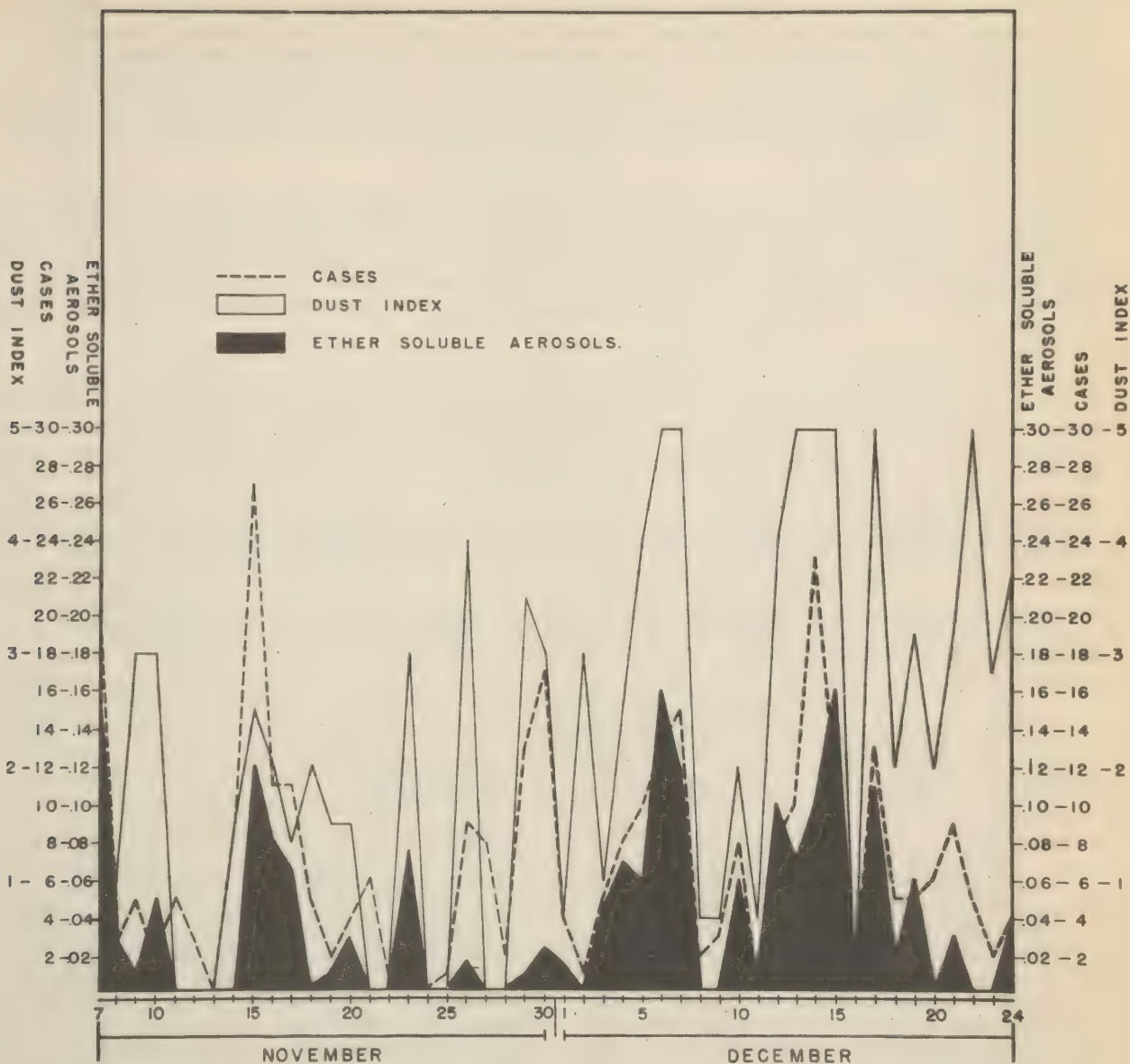


Figure 1. "Yokohama Asthma", November-December 1952. Comparison of Daily Case Incidence with Assays for Dust and Ether Soluble Aerosols

The gathering and interpretation of incidence rates leave much to be desired. Only those patients who report to Yokohama Army Hospital are considered in incidence rate and only those who complain of severe respiratory distress are listed as asthma cases. There are undoubtedly many patients reporting with minor symptoms which may be diagnosed as bronchitis, rhinitis, sinusitis, or some other respiratory disease. Many patients having once reported for treatment and finding little can be done for them do not report for treatment when again troubled by mild symptoms. The problem of incidence data is further complicated by personnel shortages. The required number of workers is not available for interview and study of each individual case. These limitations are reflected by incomplete incidence data.

Personnel limitations required air sampling to be made only from 1900 to 2400 hours daily. Therefore, the evaluation of air pollution is of necessity incomplete and the true picture of exposure is not available. Rain or strong winds often apparently cleared the air before the sampling period began. The results were low or negative for air contaminants when such may not have been the condition during most of the day.

The topography of Yokohama consists of undulating terrain and the locations from which the asthma patients come are frequently widely separated. With sufficient personnel and equipment it would be desirable to operate more sampling stations. The elimination of these inadequacies would undoubtedly result in more clearly defined results.

WATER ANALYSIS: The Water Analysis Section received 114 specimens during the year. Of these, 106 were examined for potability, 6 for use in bacteriological media, and 2 for purity (distilled water).

Methods of analysis used were those standard methods approved by the American Public Health Association (29).

Table XVI. Water Analysis Determinations

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Acidity	2	Manganese	104
Alkalinity, Methyl Orange	105	Nitrogen, Ammonium	105
Alkalinity, Phenolphthalein ...	105	Nitrogen, Nitrate	104
Aluminum	14	Nitrogen, Nitrite	104
Ammonia	1	Odor	27
Calcium	106	Oxidizing Substances	6
Carbon Dioxide	8	pH	113
Chlorides	109	Residue, Dissolved	107
Chlorine	10	Residue, Fixed	107
Color	107	Residue, Suspended	107
Fluorine	5	Residue, Total	108
Hardness, Total (Fe, Ca, Mg) ..	98	Silica	106
Heavy Metals	8	Sodium	2
Iron	14	Sulfates	105
Iron and Aluminum	104	Taste	2
Lead	4	Turbidity	106
Magnesium	104	Total	2217

In May a problem associated with the possible contamination of river water by sewerage effluent from a military camp near Sapporo, Japan was presented. Inasmuch as shipment of water samples for dissolved oxygen determination is unsatisfactory, a technician and the necessary equipment and reagents for the Winkler Method (30) for dissolved oxygen were sent to Sapporo Army Hospital in order to establish the procedure there. The continuation of the project was carried out by the personnel of the laboratory at that unit.

In June a total of 23 samples of water allegedly contaminated with gasoline and/or kerosene from base operations arrived from Johnson Air Base. Eight of these specimens were well water which were being consumed by the local Japanese population surrounding that area.

This laboratory was able to successfully establish gasoline in only 1 of these analyses. The remaining 7 samples contained the odor of a hydrocarbon resembling gasoline. This hydrocarbon, however, was not identified since it was present in such small quantities that it could not be separated from the mother liquor (water) either chemically or physically.

Fifteen samples consisting of 100 ml collections from kitchen sink drainings were also received from the same immediate area. The odor of hydrocarbon resembling kerosene was detected in these. However, as in the previous cases, because of an insufficient sample and the minute amount of hydrocarbon present, a positive identification was not possible.

The hydrocarbon which was identified had the following characteristics:

(1) Hydrocarbon (mixture)	Gasoline
(2) Specific gravity at 15.5°C.	0.7365
(3) Unsaturated hydrocarbons	Positive
(4) Burning test	Burned with a smoky flame
(5) Odor	Positive gasoline smell
(6) Boiling range	45°C to 160°C.
(7) Color (upon distillation)	Colorless

Investigation has been continued on the use and practicability of ion-exchange resins as a source of chemically pure water for medical laboratory use in the Far East. Two four-bed deionizers have been installed in the Virus and Rickettsial, and Bacteriology Departments of this laboratory and several are now in use, both in Korea and Japan, with good success. This department has recently installed a new and larger type mono-bed deionizer which, although investigations as to the purity of water obtained by its use are not yet complete, appears to be even more efficient than previous types investigated (32). Effluent water has been found to meet the requirements for distilled water as outlined in "U.S.P.", XIII (33) and the ease of operation of these units has been found a major factor in their desirability.

MISCELLANEOUS ANALYSES: The mission of Miscellaneous Analyses Section is to comply with legitimate requests which are not easily accommodated within the various other sub-sections of the Department. Requests are received from most branches of the military service in the Far East Command and cover a wide variety of procedures and analyses. (See Tables XV and XVI).

The wide variety of requests presented to the Miscellaneous Analyses Section has necessitated extensive use of the technical literature which has included a number of standard textbooks, journals, and periodicals, and a variety of supplemental books for special information regarding identification and uses of various samples submitted. Qualitative and quantitative analysis of both organic and inorganic substances has been required in many cases and the peculiar problems associated with each has required some screening of projects to assign priority to those which are the most urgent. For the identification of unknown--especially the organic compounds-- some history regarding the reason for the request is essential to handle these samples economically and not unnecessarily burden the highly trained personnel who must do this type of work. Samples submitted with the request "For Analysis" or "Identify" or some similar phrase with no justification for analysis and sometimes without the signature of the requesting officer require additional correspondence before any attempt can be made for analysis. In addition to the samples for qualitative analysis, this section also receives a variety of samples for determination of USP quality requirements of drugs and pharmaceuticals, anesthesia oxygen, insecticides, water purification tablets, medical equipment, laundry soap, and other samples, each of which may require the establishment of a new procedure and procurement of special equipment.

Table XV. Miscellaneous Specimens Received

<u>Sample</u>	<u>No.</u>	<u>Sample</u>	<u>No.</u>
Alcohol	6	Meperidine HCl	1
Alcoholic Beverages	20	Moth Balls	1
Aluminum Hydroxide	3	Neo-Arsemin	1
Atabrine	1	Opium	25
APC Tablets	1	Paint	1
Belladonna Tincture	1	pH Comparison Kit	1
Benzene Hexachloride	2	Pipettes	1
Beverages, Soft	2	Procaine	11
Calcium Hypochlorite	2	Procaine Penicillate	1
Clothing	2	Quinine Sulfate	1
Cresol	4	Quinacrine HCl	1
Emetine Hydrochloride	1	Soap, Laundry	19
Emulsifying Agent	3	Sodium Hypochlorite	1
Epinephrine Solution	16	Stopcock, 3-way	12
Fire-proofing Solution	2	Sparkling Water	1
Formalin Solution	2	Streptomycin	1
Frostbite Solution	415	Sulfobromophthalein	21
Fungicide-treated Rice Bags ...	2	Typhoid Vaccine	2
Gasoline	3	Tooth Paste	1
Glucose	4	Unknowns	74
Hair Dye	2	Urine	1
Herbs	2	Water	14
Hydrogen Peroxide	2		
Halazone	66		
Iodine Water Purification		Total	1602
Tablets	588		

Table XVI. Determinations Completed by Miscellaneous Analyses

<u>Qualitative</u>	<u>No.</u>	<u>Qualitative</u>	<u>No.</u>
Acetaldehyde	2	Borax	1
Acetone	4	Boric Acid	2
Acetylsalicylic Acid	8	Bromural	1
Acids	75	Cadmium	2
Acrolein	2	Calcium	4
Alcohol, Ethyl	3	Caffeine	1
Alcohol, Methyl	2	Carbon Dioxide	73
Aldehydes	16	Carbohydrates	1
Alkalies	73	Carbon Monoxide	73
Alkaloids	54	Carbonizable Matter	6
Alkyl Sulfides	1	Chlorides	10
Aluminum	3	Chlorine	1
Aluminum Hydroxide	3	Chloral Hydrate	14
Aluminum Silicate	1	Codeine	3
Arsphenamine, Sodium	1	Copper	1
Arsenic	8	Cyanide	23
Artificial Coloring	13	DDT	1
Atropine	1	Dibutyl Phthalate	2
Aureomycin	6	5, 5 Diphenylhydantoin	2
Barbiturates	15	Epinephrine	16
Barium	2	Ethyl Salicylate	1
Benzene Hexachloride	2	Formaldehyde	5
Benzoates	2	Fusel Oil	12
Benzoic Acid	2	Glucose	2

Table XVI. Determinations Completed by Miscellaneous Analyses

Qualitative	No.	Qualitative	No.
Glycerine	2	Sulfides	4
Halazone	1	Sulfites	4
Halogenated Compounds	73	Sulfonamides	6
Halogens	5	Sulfur	7
Heavy Metals	29	Systematic Qualitative Analy-	
Heroin	1	sis	1
Hippurates	2	Undecylinic Acid	1
Hydrocarbons	10	Vegetable Oil	1
Hydrogen Peroxide	4	Volatile Matter	4
Hyoscyamine	1	Zinc	1
Inorganic Salts	2		
Iron	5		
Iron Oxide	1	Total	954
Ketones	21		
Lactose	2	Quantitative	No.
Lead	1	Acidity	4
Magnesium	2	Acid, Free	3
3 Methyl, 5,5 Ethyl Hyndantoin .	2	Acid Value	2
Mercurochrome	1	Alcohol, Ethyl	106
Mercury	2	Alcohol, Methyl	19
Methyl Salicylate	1	Aldehydes	5
Meperidine	1	Alkali, Free	6
Merthiolate	1	Aluminum Hydroxide	3
Methamphetamine	2	Arsenic	4
Methanol	14	Atabrine	1
Monochloroacetic Acid	2	Belladonna Assay	1
Morphine	5	Chloride	10
Naphthalene	1	Chlorine	89
Nitrogen	6	Cresol Assay	4
p-Nitrophenol	1	Dextrin	4
Nupercaine	2	Dibutyl Phthalate	1
Organic Impurities	8	Distillation, Fractional	5
Oxidizing Substances	73	Emetine Hydrochloride	1
Phenacetin	1	Esters	3
Phenols	18	Ethylene Glycol	2
Procaine Hydrochloride	7	Fluorides	5
Quinacrine	2	Formaldehyde	2
Quinidine	2	Glucose	64
Quinine Hydrochloride	1	Hydrogen Peroxide	2
Reducing Sugars	6	Iodine	588
Salicylanilide	3	Iodine Value	4
Salicylic Acid	2	Iron Oxide	1
Scopolia	1	Loss on Drying	8
Silicate	3	Matter Insoluble in Alcohol ..	15
Soap	1	Matter Volatile at 105°C.	15
Sodium	8	Meperidine	1
Sodium Bicarbonate	3	Morphine	2
Sodium Carbonate	1	Non-volatile Residue	13
Starch	2	Opium Assay	2
Sucrose	1	Oxygen	255
Sulfobromophthalein	2	p-Nitrophenol	1
Sulfadiazine	8	Phenol	2
Sulfaguanidine	1	Procaine Assay	95
Sulfasoxazole	1	Quinacrine	3
Sulfate	6	Quinine	1
Sulfathiazole	5		

Table XVI. Determinations Completed by Miscellaneous Analyses Cont.

<u>Quantitative</u>	<u>No.</u>	<u>Physical Constants</u>	<u>No.</u>
Residue on Ignition	4	Solubility in Water	12
Rosin	11	Specific Gravity	5
Soap, Anhydrous	11	Taste	5
Sulfobromophthalein	5		
Soluble Starch	4		
Sucrose	3	Total	98
Sulfate	4		
Sulfadiazine	5		
		<u>Miscellaneous</u>	<u>No.</u>
Total	1435	Chemical Extractions	8
		Chromatographic Analysis	7
<u>Physical Constants</u>	<u>No.</u>	Emulsifying Power	10
Boiling Point	2	Fish Toxicity	6
Color	4	Microscopic Examination of	
Melting Point	25	Crystals	6
Odor	5	Mouse Toxicity Tests	120
Reaction, pH	27	Pipette Calibration	7
Refractive Index	3	Pressure Check, 3-way Stopcock	36
Solubility in Alcohol	12	Preparation Organic Deriv-	
		atives	1
		Spectrographic Analysis	1
		Synthesis, Salicylanilide	1
		Taxonomic Identification	13
		Total	216
		Total Miscellaneous Analyses	2703

Drugs and pharmaceuticals are checked fully for USP (34) and USD (35) requirements as are also anesthesia oxygen and other substances which will be used by the Medical Department. Alcoholic beverages and refreshment items are checked against the requirements of "A.O.A.C." (36).

In those cases where toxicity may be a question, the use of oral (ad libitum) feeding and intraperitoneal injection of laboratory animals has been a useful and valuable procedure.

A total of 63 unknowns were examined during the year. Sixty of these were positively identified and in the remaining cases the requirements of the request could be satisfactorily complied with. Samples of suspected narcotics when identified included the following: Borax, DDT powder, calcium hypochlorite ampules, barbiturates, heroin, opium, scouring powder, mercuric sulfide, procaine, sodium bicarbonate, starch, mephesin, APC tablets, and codeine. In addition, the following unknowns were identified: Quinine, sodium hypochlorite, nupercaine, quinidine, codeine, sulfadiazine, dilantin sodium, methyl testosterone, component parts of a captured enemy medical kit, cement residue in Coca-Cola bottle, and methamphetamine.

Quality control tests were run on anesthesia oxygen, clothing impregnant, laundry soap, alcoholic beverages, and water purification tablets of both halazone and iodine types.

Pharmaceutical assays were required on several products, including the Frost-bite Treatment Solution described in 1951 (37), hydrogen peroxide, sulfobromophthalein, typhoid vaccine, procaine hydrochloride, emetine, belladonna extract, and others.

INVESTIGATIONS: HEMORRHAGIC FEVER: This laboratory has furnished equipment, personnel, and services in cooperation with the laboratory at the Hemorrhagic Fever Treatment Center in Korea in order to supplement the clinical investigations of the physicians studying the physiology of hemorrhagic fever. Inasmuch as the laboratory in Korea was operating most of the past years in tents it was found expedient to establish a regular system for air courier service for specimens from Korea. The same courier system was used for the sending of equipment and stock reagents to Korea. Four specimen containers of approximate suitcase size were constructed to accommodate a test tube rack, 250 ml specimen bottles, and a dry ice refrigeration compartment. This case was found satisfactory for transfer of urine specimens, plasma samples, and reagents between the two laboratories and, since it could be hand carried by the courier, breakage of samples was practically eliminated. Stock reagents made by the clinical chemistry section of the 406th Medical General Laboratory were furnished routinely for most of the procedures which required precise weighing of standards. Assistance was furnished in investigation of the following:

Cortins and 17-Ketosteroids - Cortins (formaldehydogenic substances) were determined by the method previously described in this report (2) and the 17-ketosteroids total was determined after the method of Holtorff and Koch (38). Determination of the cortins generally showed an elevated value which increased through the febrile and oliguric phases to reach a maximum at the second to fifth day of diuresis. This value was most commonly twice normal but on occasion an eight to tenfold increase over normal was noted. These values rapidly returned to normal, and remained normal, in convalescence. The 17-ketosteroids opposed the cortins increase. Determinations showed a low total of 17-ketosteroids (2-6 milligrams per 24 hours) in the febrile and anuric phase and a return to normal at about the same time shown by the cortins. With the 17-ketosteroids there were occasional erratic increases to 40-50 milligrams per 24 hours during the convalescent period. Samples of two consecutive 24-hour collections of urine taken immediately prior to the discharge of the patient from the hospital showed that both the cortins and 17-ketosteroids were in the normal range at that time. Butanol extracts of urine aliquots have been prepared according to a method suggested by Reddy, Jenkins, and Thorn (39) and are stored pending arrival of suitable standards which will allow fractionation of the 17-ketosteroids into the alpha and beta components. The correlation of these laboratory data with the clinical conditions of the patient and eosinophile counts is being done by the professional staff of the Hemorrhagic Fever Treatment Center in Korea.

Potassium and Sodium - Serum potassium and sodium were found to be normal in the early stages of hemorrhagic fever and both showed a tendency to increase during oliguria and even in early diuresis. Urine potassium concentration and excretion was commensurate with blood levels and urine outputs, but the urinary concentration of sodium was low (3-25 mEq per liter) in early diuresis. This was found to increase approximately five or six days following increased urine output. Total 24 hour urine sodium in some patients voiding as much as six to eight liters of urine was in the vicinity of 120-130 mEq/liter.

Chloride - Serum chloride values paralleled the sodium changes in most cases and the same could be said for the urine concentrations and total 24-hour elimination.

Carbon Dioxide and Blood pH - The carbon dioxide combining power and blood pH were found to be essentially normal in all stages of the disease. Even in some very seriously ill patients the blood pH was found to be in the range of pH 7.38 to 7.52.

Calcium - Serum calcium was in the range of 3.4 to 4.6 mEq per liter in most patients during the febrile, anuric, and early diuretic phases. These values tended to increase to approximately 5 to 5.5 mEq/liter in the recovery phase. Urine calcium concentrations were in the range of 0.5 to 1.0 mEq per liter during oliguria but increased to 1.0 to 1.5 mEq in diuresis and remained so during convalescence.

Phosphate, Inorganic - Serum inorganic phosphate was either normal or slightly elevated in the milder cases, but in the patients with urine outputs less than 500 ml per 24 hours the serum phosphate increased to as much as 12 mEq per liter in some

cases. This value dropped abruptly when urine flow was restored and was generally less than 5 mEq per liter within 3 to 4 days following diuresis.

Histamine Studies of EHF - The use of certain antihistaminics in the treatment of hemorrhagic fever was suggested and this laboratory attempted to establish a suitable chemical or biological procedure for determination of histamine in blood plasma. The method of Lubschez (39) for chemical assay was investigated and discarded for various reasons. Improperly purified reagents, unfavorable recovery studies, and other technical difficulties with the chemical method prompted the use of biologic assay after the method of Code (40). The use of the kymograph and guinea pig ileum proved the more satisfactory, but with the facilities of this laboratory no method was found to be entirely satisfactory for histamine. Results of the examination of plasma samples, quick frozen in Korea and shipped in dry ice to Japan for assay failed to indicate any increase in histamine content of the plasma in any of the 27 patients examined.

Electrophoretic Studies - In July an American Instrument Company Electrophoresis unit was procured from the Atomic Bomb Casualty Commission in Hiroshima. Air conditioning was installed in one small room of the laboratory and the unit placed in operation the latter part of August. To date, over 120 specimens of plasma and serum have been analyzed on the instrument. Results gained on normal specimens compare favorably with those reported in the literature, indicating proper operation of the instrument.

Presently the instrument is devoted to full time operation on specimens received from Korea in connection with the investigation of hemorrhagic fever. One of the early difficulties encountered was the receipt of improperly refrigerated specimens which contained considerable bacterial growth. Another problem was the high lipid content of some specimens which resulted in a falsely high beta globulin fraction. The refrigeration problem has been solved by use of the shipping boxes previously described and a high speed centrifuge has been found satisfactory for removal of lipids and clarification of serum or plasma without the loss of protein-bound lipids which are present in true solution. Only the results gained with the improved technic are reported. Plasma for electrophoretic studies was collected on patients daily, with occasional skipping, from admission to 4 or 5 days after diuresis. The plasma was separated immediately, quick frozen in dry ice, and shipped in dry ice to this laboratory. All samples were kept in the "deep freeze" until thawed at room temperature for analysis. Control samples on healthy persons handled in the same manner were collected and submitted. Scudder (41) reports that plasma may be stored in the frozen state (-20°C) and thawed at 37°C without alteration of the electrophoretic pattern. Fasting plasma specimens are subjected to high speed centrifugation and the clear plasma is diluted 4:10 with barbiturate buffer at pH 8.6 and an ionic strength of 0.2. This is placed in a bag of Visking tubing and dialyzed against a liter of the same buffer with mechanical stirring in a specially constructed dialyzer (42) at about 4°C for 16 hours. The standard clinical cell was used in each experiment. The electrophoresis diagrams were recorded on photographic plates and the plates evaluated in the usual manner by enlargement through projection, tracing by hand and planimetry. The relative concentration of each component was computed from the size of its area as compared to the total area under the curve. Calculations were made on the ascending boundaries in all cases. Normal plasma was interposed at regular intervals to check operation of the machine.

Results of examination of 6 mild to moderate cases of hemorrhagic fever are reported in Table XVII. Selection of patients was done on admission to the hospital as confirmed cases and evaluation of severity was necessary in retrospect. No severe cases are included in this series since none of the patients selected fell into that category. Concentration of the protein fractions are shown in grams per 100 ml of plasma and as the ratio "r" of the component area to total area of the electrophoretic diagram. Table XVII gives the average figure for the total number of days specimens were collected.

The mean of total samples examined in each case shows a consistently low albumin/globulin ratio and an elevated alpha globulin fraction. The fibrinogen, beta

Table XVII. Mean Electrophoretic Fractions of Sera of Hemorrhagic Fever Patients

Case	No. Spec.	Albumin		Alpha 1 Globulin		Alpha 2 Globulin		Beta Globulin		Fibrinogen		Gamma Globulin		A/G
		r	Gm per 100 ml	r	Gm per 100 ml	r	Gm per 100 ml	r	Gm per 100 ml	r	Gm per 100 ml	r	Gm per 100 ml	
A	10	0.424	3.18	0.087	0.65	0.124	0.93	0.120	0.91	0.072	0.52	0.173	1.30	0.74
B	19	0.398	2.84	0.090	0.64	0.122	0.90	0.136	0.97	0.077	0.55	0.187	1.35	0.67
C	10	0.427	3.00	0.079	0.56	0.116	0.81	0.143	1.00	0.086	0.59	0.151	1.07	0.74
D	13	0.457	3.31	0.088	0.63	0.101	0.77	0.125	0.86	0.069	1.25	0.171	0.51	0.85
E	12	0.420	3.12	0.092	0.68	0.133	0.98	0.126	0.93	0.072	0.53	0.156	1.15	0.74
F	10	0.492	3.67	0.086	0.64	0.125	0.93	0.118	0.88	0.055	0.41	0.124	0.93	0.98
Norm	15	0.603	4.04	0.046	0.31	0.072	0.48	0.121	0.81	0.051	0.34	0.110	0.74	1.53

globulin, and gamma globulin fractions do not show any marked response and their respective increase was found to approximate the percentage drop in the albumin. None of the individual samples examined were found to show the marked increase expected in antigen-antibody response for gamma globulin and the increase in the alpha globulin components is thought to be due to the tissue destruction and fever which occur early in this disease. Stern and Reiner (43) report that diseases accompanied by tissue destruction and fever show significant rises in the alpha globulin component.

Individual specimens examined on each patient showed a progressive decrease in the albumin component in all cases until a minimum was reached between the ninth and fifteenth day of the disease, at which time the albumin fraction was noted to increase slowly. Selected days to show this trend are outlined in Table XVIII. It will be noted that in all cases the A/G ratio was decreased for the entire range of examinations. Dole and Braun (44) report their mean for albumin-globulin ratio determined electrophoretically to be near 1.5 for normal patients. This is approximately 30% lower than values obtained by chemical fractionation of the same plasma samples.

It is not known at what time the decreased A/G ratio occurs or when it returns to normal, but an attempt is being made to study some of the convalescent patients to establish this.

Study of Bank Blood Available in Korea - This study was undertaken in August, September, and October 1952, in order to obtain additional data concerning the properties of the stored whole blood available for transfusion in the combat zone. Since most of the blood is shipped from the United States the most extensive effort was directed toward studying blood from that source. Comparable studies are reported from the 406th Medical General Laboratory and from the Surgical Research Team in Korea. It is expected that the same data will be available from similar work in the United States. These data are supplemented by an additional series similarly describing blood obtained in Japan.

In the study of ZI blood an effort was made to analyze samples of 10 bloods on alternate days after drawing. Such an extensive group was expected to provide a statistical background for the other studies undertaken. Because of the less flexible supply of blood in Korea, varying numbers of samples were taken, as indicated in Table XIX.

When this study was near completion it was learned that preservative solutions of different composition were used at different times. It was impossible to determine the

Table XVIII. Variation of Plasma Protein Fractions During Hemorrhagic Fever

Patient	Day of Illness	Total Protein	Albumin	Alpha 1	Alpha 2	Beta	Gamma	Fibrinogen	A/G Ratio
A	6	6.67	0.419	0.105	0.143	0.097	0.147	0.089	0.72
	10	7.84	0.391	0.097	0.128	0.125	0.180	0.079	0.64
	15	8.03	0.470	0.071	0.114	0.142	0.167	0.036	0.89
B	4	6.28	0.492	0.088	0.102	0.144	0.112	0.062	0.97
	15	6.86	0.345	0.081	0.129	0.137	0.250	0.058	0.53
	21	8.42	0.382	0.082	0.160	0.107	0.196	0.073	0.62
C	7	6.28	0.413	0.088	0.142	0.144	0.120	0.093	0.72
	14	7.45	0.413	0.070	0.127	0.123	0.178	0.099	0.70
	18	7.45	0.446	0.079	0.091	0.132	0.175	0.077	0.81
D	3	6.28	0.500	0.090	0.101	0.132	0.129	0.048	1.00
	10	7.84	0.389	0.087	0.106	0.114	0.217	0.087	0.64
	16	7.25	0.500	0.055	0.074	0.143	0.168	0.060	1.00
E	5	7.45	0.456	0.120	0.143	0.121	0.087	0.073	0.83
	10	6.28	0.373	0.106	0.143	0.130	0.174	0.074	0.60
	16	7.45	0.460	0.076	0.121	0.124	0.151	0.068	0.85
F	6	7.65	0.487	0.067	0.133	0.130	0.130	0.053	0.95
	9	7.45	0.470	0.088	0.128	0.126	0.122	0.066	0.89
	16	7.45	0.490	0.093	0.123	0.123	0.127	0.044	0.96
G	8	6.10	0.448	0.099	0.106	0.190	0.097	0.060	0.81
	12	6.66	0.397	0.108	0.101	0.227	0.118	0.049	0.66
	21	7.82	0.484	0.096	0.071	0.167	0.115	0.067	0.94
H	3	7.45	0.491	0.081	0.090	0.165	0.113	0.061	0.96
	11	8.00	0.434	0.092	0.126	0.118	0.145	0.085	0.77
	20	7.65	0.460	0.067	0.085	0.228	0.094	0.069	0.85
Normal		6 - 8	0.603	0.046	0.072	0.121	0.110	0.051	1.53

Clinical Notes:

Patient	Fever*	Oliguria**	Onset Diuresis***	Remarks
A	Thru 6th day	None	Began 9th day	Mild case
B	Thru 6th day	None	Began 10th day	Moderate case
C	Thru 4th day	None	Began 8th day	Mild Case
D	Thru 5th day	None	Began 11th day	Moderate case
E	Thru 4th day	None	Began 8th day	Moderate case
F	Thru 6th day	None	Began 5th day	Mild case
G	Thru 9th day	Present	Began 14th day	Moderate case
H	Thru 4th day	None	Began 7th day	Moderate case

* Fever range from 102°- 104°F.

** Less than 700 ml urine per 24 hours.

*** Abrupt increase in urine to 3-8 liters per 24 hours.

solution used in any specific sample and since samples tended to be taken in groups it is probable that the distribution of the various preservatives is not a random one. Accordingly, the data presented is subject to a possible systematic error on this basis. In

Table XIX. Plasma Potassium and Hemoglobin in Banked Blood

Age (Days)	No. Samples			Plasma Potassium (mEq/l)						Plasma Hemoglobin (mg/100 ml)					
	A	B	C	A		B		C		A		B		C	
				x	a	x	a	x	a	x	a	x	a	x	a
0	--	--	10	----	----	----	----	5.6	1.8	----	----	----	----	42	14
2	--	--	10	----	----	----	----	8.1	2.7	----	----	----	----	112	95
4	--	--	10	----	----	----	----	8.9	2.5	----	----	----	----	69	8
5	10	--	--	9.1	1.5	----	----	----	----	16	7	----	----	--	--
6	10	--	10	10.9	2.5	----	----	11.3	1.8	17	6	----	----	33	68
7	--	5	--	----	----	9.9	----	----	----	----	----	52	----	--	--
8	10	--	10	9.5	1.8	----	----	13.1	1.4	38	36	----	----	60	23
9	--	2	--	----	----	11.2	----	----	----	----	----	76	----	--	--
10	10	--	10	11.9	3.5	----	----	14.9	2.1	30	20	----	----	62	52
11	--	13	--	----	----	14.0	2.0	----	----	----	----	61	60	--	--
12	10	--	10	13.0	1.3	----	----	15.7	1.3	23	13	----	----	120	41
13	--	13	--	----	----	14.5	2.3	----	----	----	----	68	21	----	----
14	10	--	10	15.5	3.0	----	----	14.6	2.4	62	39	----	----	71	26
15	--	10	--	----	----	16.5	1.4	----	----	----	----	66	50	----	----
16	10	--	10	19.4	6.6	----	----	15.8	2.0	134	104	----	----	94	44
17	--	13	--	----	----	16.6	2.9	----	----	----	----	43	----	----	----
18	10	--	10	18.3	2.7	----	----	16.4	2.0	83	58	----	----	91	43
19	--	15	--	----	----	18.2	1.6	----	----	----	----	87	----	----	----
20	10	--	10	19.4	4.0	----	----	15.4	1.5	143	67	----	----	111	39
21	--	11	--	----	----	19.0	2.3	----	----	----	----	113	----	----	----
22	10	--	10	18.3	3.5	----	----	18.7	1.7	79	74	48	----	121	38
24	10	--	10	20.6	2.0	----	----	19.4	1.8	79	----	----	----	145	55
25	--	11	--	----	----	20.3	----	----	----	----	----	142	----	----	----
26	10	--	10	19.2	2.3	----	----	22.2	1.6	79	69	----	----	187	40
28	10	--	10	19.7	2.1	----	----	20.1	1.6	108	----	----	----	183	44
30	10	--	10	23.4	3.4	----	----	19.6	1.8	194	70	----	----	207	36

x - Mean

a - Standard Deviation

A - U. S. Blood, studied in Tokyo

B - U. S. Blood, studied in Korea

C - Japan collected blood, studied in Tokyo

view of the regularity and narrow distribution of the plasma potassium levels and, to a lesser extent, of the plasma hemoglobin these data are presented with the above reservation. A similar series of whole blood glucose levels is not recorded here because of an apparently very marked effect of the different solutions on this factor. Attempts to correlate the various indices observed without knowledge of this uncontrolled variable is considered unwarranted.

Methods - Blood bottles for sampling were selected at random from stocks available and mixed for five minutes by gently rolling the bottle from side to side. The mixed bottle was sampled using a size 17 needle and a dry syringe. The hematocrit was determined and the plasma separated for analysis immediately.

Plasma potassium was determined by flame photometry using an internal lithium standard. For convenience the analyses done in Korea were on plasma samples stored for three to four weeks after they were drawn from the bottle of blood, but control samples demonstrated no detectable change in potassium concentration during that time.

Plasma hemoglobin was determined by the method of Bing et al, as modified at the 406th Medical General Laboratory (45).

Into a suitable colorimeter cuvette are placed, in sequence, 0.6 cc of 2% benzidine base in glacial acetic acid, 0.2 cc of the suitably diluted sample and 0.2 cc of 0.6% hydrogen peroxide. After standing one hour 6 cc of 20% acetic acid are added, and the color intensity read at wave length 640 mu. Standards of 5, 7.5, and 10 mg% were prepared by diluting whole blood and the unknowns were diluted to read within that range. A Coleman Junior Spectrophotometer was used in these studies.

Since there was a frequent interfering cloudiness in the low dilutions of plasma it has occasionally been necessary to express a concentration as being below that comparable to the lowest dilution that failed to give the cloudiness though the apparent color intensity was below that of the lowest standard.

The red cell osmotic fragility curve was determined in phosphate buffered saline solutions according to the method of Parpart et al (46). It has been suggested by Sack et al (47) that the 0.6% saline fragility most closely parallels in vivo survival times. This study was arranged to cover that range. All necessary reagents were prepared as a single lot at the 406th Medical General Laboratory for uniformity.

Studies of Blood from the United States - This group consists of blood collected in bottles using ACD solutions obtained from the regular shipments to the Far East Command.

The plasma potassium and hemoglobin are presented in Table XIX. There is a regular, almost linear rise of the potassium with time, which may be extrapolated to 4.0 mEq/liter at the time of drawing and reaches approximately 20.0 mEq/liter on the twentieth day. Thereafter, the rise is slower, reaching a level of 24 mEq/l on the 30th day. There is a relatively small scatter of individual specimens about the mean.

There was a very much wider scatter of individual observations of the plasma hemoglobins, but the level appears to rise irregularly from 10-15 mg percent on the fifth day to 100 mg percent on the twentieth day.

The plasma potassium and hemoglobin levels of individual specimens showed only approximate correlation with occasional marked exceptions.

As with the plasma hemoglobin, there is a wide range of variation among the red blood cell fragility curves. The mean percent hemolysis for each saline concentration is presented in Tables XX and XXI.

A group of 10 has not proven large enough to allow evaluation of minor changes. It is apparent, however, that after 5 days there is little change until the 16th or 17th day when a regular shift toward increasing fragility starts.

Discussion - In presenting this material it is recognized that the data can be considered only as indirect indices of quality. Neither increased plasma potassium nor increased plasma hemoglobin per se can be considered evidence of deterioration from the point of view of usefulness in transfusion since the correlation of toxicity at such plasma levels is unknown and, of more importance, it is possible that considerably more of these materials are released into the circulation by the breakdown of cells following transfusion. If 10% of the red cells of blood typical of this study hemolyze shortly after transfusion, the potassium released would approximate that of the transfused plasma (2-3 mEq per liter of blood) while the plasma hemoglobin would be exceeded thirtyfold (14.5 gm vs 0.5 gm per liter of blood). No final judgment as to preserving qualities or usefulness of blood is offered herein. Presumptive evidence is presented but only after these data are correlated with red blood cell survival studies presently being carried out by the Surgical Research Team can a definitive decision be made on these points.

Paper Chromatography of the Opium Alkaloids - The difficulties attending the purification and identification of opium alkaloids in tissues and body fluids

Table XX. Mean Osmotic Fragility, U. S. Blood

Studied in Tokyo

Age (Days)	Saline Concentration vs % Hemolysis				
	0.7	0.6	0.55	0.4	0.25
5	1	8	31	88	96
6	0	3	13	88	94
8	0	4	18	86	90
10	0	10	26	92	95
12	1	11	23	86	94
14	1	11	30	91	95
16	2	10	14	92	96
18	4	17	34	90	95
20	10	23	36	91	94
22	11	28	51	86	95
24	10	26	44	88	93
26	17	40	62	91	96
28	10	23	42	90	95
30	18	38	54	91	96

Studied in Korea

Age (Days)	Saline Concentration vs % Hemolysis					
	0.6	0.55	0.5	0.45	0.4	0.3
7	3	9	26	75	92	98
11	7	16	41	82	94	97
13	8	22	40	80	90	97
15	6	12	31	64	87	95
17	8	22	43	73	91	97
19	17	32	57	84	93	96
21	7	23	46	74	91	97
25	12	35	59	86	94	98

Table XXI. Mean Osmotic Fragility, Japan Collected Blood Studied in Tokyo

Age (Days)	Saline Concentration vs % Hemolysis				
	0.7	0.6	0.55	0.4	0.25
0	1	7	26	85	94
2	1	8	33	91	95
4	1	11	41	94	97
6	2	12	38	89	94
8	1	17	39	89	95
10	2	17	48	92	96
12	4	22	42	93	97
14	5	21	45	91	96
16	4	23	47	93	97
18	7	24	52	94	97
20	15	41	65	89	94
22	15	40	61	86	92
24	9	27	55	90	95
26	9	37	57	93	96
28	20	46	73	91	95
30	17	44	66	92	95

have long plagued the toxicologist. The amounts of alkaloids present in toxicologic specimens are extremely small and must be isolated in a relatively high state of purity before chemical identifications can be made. Because the isolation technics are imperfect and the chemical tests subtle, the experience and skill of the toxicologist is often the deciding factor between a positive and negative alkaloid identification. Since the finding of narcotic alkaloids raises questions of grave nature, there is a reluctance to report as positive any but the most conclusive of the chemical tests; doubtful or borderline reactions are reported as negative. The application of the paper chromatography technic has done much to minimize these difficulties.

The general methods and principles of paper partition chromatography can be described briefly as follows: A small drop containing the solute under investigation is placed near one edge of a strip or sheet of filter paper. After the spot has dried the paper is placed in a sealed container. The atmosphere within the chamber consists of air saturated with water and the vapors from an appropriate solvent. After the paper has come to equilibrium with the atmosphere the end of the paper containing the spot is brought into contact with the solvent. The solvent flows past the spot by capillary action and up the length of the paper. At this time the solute will have moved a distance along the paper, that distance depending upon the relative solubility of the solute between water and the solvent (i.e., the partition coefficient). The movement of the solute zone has been explained ⁽⁴⁸⁾ conveniently as follows: "The cellulose fibers have a strong affinity for the water present in the solvent phase but very little for the organic liquid. The paper itself is thought of as an inert support holding a stationary aqueous phase. As solvent flows through a section of the paper containing the solute a partition of this compound occurs between the mobile organic phase and the stationary water phase. Thus, some of the solute leaves the paper and enters the organic phase. When the mobile liquid reaches a section of the paper containing no solute, partition again occurs. This time, solute is transferred from the organic phase to the paper phase. With continuous flow of solvent, the effect of this partition between the two phases is the transfer of a solute from the point of its application on the paper to a point some distance along the paper in the direction of solvent flow."

(1) General Procedure - Rectangular sheets of filter paper measuring 28 x 32 cm are cut from larger sheets of No. 1 Whatman paper. A pencil line (base line) is drawn 4 cm from the longer edge of the paper, paralleling that edge. On points along this line, at 3 cm intervals, 0.005 to 0.01 ml drops of the samples to be chromatographed are placed using 0.1 ml Mohr pipettes. The paper is dried at room temperature. When volumes greater than 0.01 ml are to be chromatographed, the solution is applied to the paper in aliquots, allowing each aliquot to dry before further additions are made. The paper is then bent perpendicularly to the base line into a cylinder and fastened at the end opposite the base line with paper clips using a paper bridge to separate the edges which close the cylinder. The paper cylinder is lowered, base line end downward, into a Smillie jar (Figure 2). The jar is clamped shut, using stopcock grease to effect an air-tight seal. Employing a pipette, the aqueous phase of the two-phase, water-solvent mixture (cf. below) is introduced through opening A onto cotton surrounding delivery tube B. This arrangement facilitates saturation of the atmosphere. Enough solution is applied to saturate the cotton, any excess falling into Beaker C. Opening A is stoppered and the apparatus is untouched for a period of four or five hours during which time the paper reaches equilibrium with the enclosed atmosphere. At the end of this period, 50 ml of the solvent phase of the two-phase mixture is introduced into the bottom of the jar via funnel D and delivery tube B. The solvent immediately begins its capillary climb up the paper. After approximately 13 hours the solvent front has advanced to within 2 or 3 cm of the top of the paper cylinder. The paper is then removed and laid flat and the solvent front boundary is marked immediately with pencil. The paper is air dried at room temperature by clipping to an electric fan. Using an atomizer, the paper is sprayed with a reagent to show the location of the alkaloid areas. In establishing the R_f value a point is selected in the alkaloid spot representing the greatest color density. The distance between this point and the base line divided by the distance between the base line and the solvent front boundary is the R_f value of that spot.

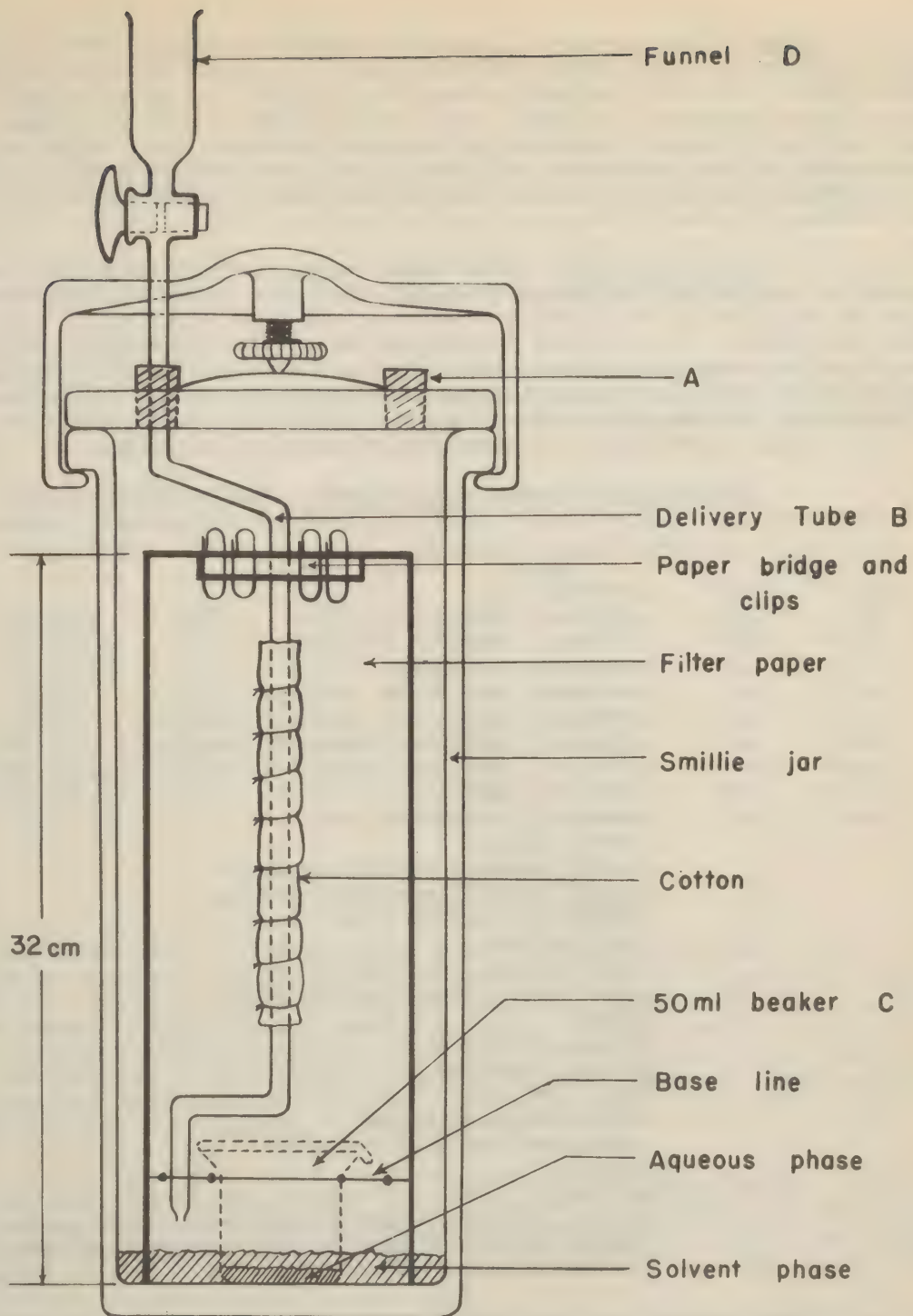


Figure 2. Chromatographic Apparatus

(2) Paper Chromatography of Pure Opium Alkaloids - Before toxicology studies could be initiated, it was necessary to establish a workable procedure using pure alkaloids. While more than 25 alkaloids have been isolated from opium, about 98% of the total alkaloid content of opium can be accounted for by 6 of these--namely, morphine, narcotine, papaverine, thebaine, codeine, and narceine. Accordingly, of the naturally-occurring opium alkaloids, only these 6 compounds were considered in this study. In addition, 3 synthetic morphine derivatives were included in the series: heroine, dionine, and apomorphine.

Stock solutions - Stock solutions of the 9 alkaloids and their hydrochloric and acetic acid salts were prepared. The free bases were made up as 0.50% solutions and the salts in concentrations equivalent to 0.50% solutions of their corresponding free bases. Fifty percent ethanol was employed as the solvent for all of the alkaloid salts except narcotine acetate, which was made up with 95% ethanol. Of the free bases, narcotine, thebaine, heroine, and apomorphine were dissolved in chloroform; papaverine, codeine, and dionine were prepared in 50% ethanol; morphine was made up with isoamyl alcohol; and narceine with 10% ammonium hydroxide.

Chromatographic developing solvents - The following solvents were employed experimentally in an attempt to find a suitable chromatographic developing agent:

n-butanol
n-butanol, acetic acid
n-butanol, ammonium hydroxide
n-butanol, chloroform
n-butanol, chloroform, acetic acid
n-butanol, chloroform, ammonium hydroxide
n-butanol, saturated sodium bicarbonate
n-butanol, phosphate buffer (serial pHs from 4.5 to 8.5)
n-butanol, hydrochloric acid
n-butanol, sodium hydroxide
isoamyl alcohol
isoamyl alcohol, acetic acid
isoamyl alcohol, ammonium hydroxide
isoamyl alcohol, pyridine
butyl acetate
butyl acetate, acetic acid
butyl acetate, ammonium hydroxide
toluene
toluene, acetic acid
toluene, ammonium hydroxide
chloroform
chloroform, acetic acid
chloroform, ammonium hydroxide
pyridine
aniline
ethanol (various concentrations) acetic acid
ethanol (various concentrations), ammonium hydroxide
ethylene glycol monoethyl ether

Of these, only butanol-acetic acid and isoamyl-acetic acid gave well-positioned, clear-cut separations. Because its slower capillary climb up the paper is more suitable for laboratory time arrangements, the isoamyl alcohol solvent was selected in preference to the butanol solvent. It is prepared as follows: isoamyl alcohol (100 ml), glacial acetic acid (10 ml), and water (50 ml) are shaken together thoroughly in a separatory funnel and the mixture allowed to stand for 3 days at room temperature before being separated into solvent and aqueous phases. The 3-day standing period enables the solutions to come to equilibrium with respect to the formation of isoamyl acetate.

Spot developing reagents - Preliminary trials with a number of reagents giving color reactions with opium alkaloids led to the selection of a modified

Dragendorff's reagent and a potassium iodoplatinate solution as the reagents most nearly filling the requirements of this study. They are prepared in the following manner:

Dragendorff's reagent: Bismuth subnitrate, 2.5 gm, water, 20 ml, glacial acetic acid, 5 ml, and potassium iodide (4 gm previously dissolved in 10 ml of water) are mixed. The precipitate that forms on standing for about 3 hours is removed by filtration. The filtrate is the stock solution. Just before using, one part of stock solution is mixed with 2 parts of glacial acetic acid and 3 parts of water.

Potassium iodoplatinate reagent: 1 ml of 10% platinic chloride and 25 ml of 4% potassium iodide are mixed and the resulting solution made up to 50 ml with water.

The sensitivities of these two reagents were tested against 10, 20, and 50 microgram spots of the 9 alkaloids which had been developed chromatographically as described above. All quantities of each of the alkaloids were applied to the paper in 0.01 ml volumes. The results are shown in Table XXII.

Table XXII. Reagent Sensitivity for Alkaloids

Alkaloid	Quantity of Alkaloid					
	10 mu		20 mu		50 mu	
	D ¹	P ²	D	P	D	P
Morphine	Minus	Plus	Minus	Plus	Plus	Plus
Narcotine	Plus	Minus	Plus	Plus	Plus	Plus
Papaverine	Plus	Plus	Plus	Plus	Plus	Plus
Thebaine	Plus	Plus	Plus	Plus	Plus	Plus
Codeine	Plus	Plus	Plus	Plus	Plus	Plus
Narceine	Minus	Minus	Minus	Minus	Plus	Plus
Heroine	Plus	Plus	Plus	Plus	Plus	Plus
Dionine	Plus	Plus	Plus	Plus	Plus	Plus
Apomorphine	Minus	Plus	Plus	Plus	Plus	Plus

1 Dragendorff's reagent

2 Potassium iodoplatinate reagent

It is seen from the table that when potassium iodoplatinate was used all of the alkaloids but narcotine and narceine could be detected in 10 microgram quantities, and that the narcotine spot was visible at the 20 microgram level. With Dragendorff's reagent, morphine, narceine, and apomorphine could not be discerned at a 10 microgram level. Fifty microgram spots of all alkaloids were visible when either reagent was used. The platinate is the reagent of choice because it offers a better contrast between spot and background and is more sensitive in its reaction with morphine, the alkaloid of greatest interest in this laboratory.

R_f values: The 9 opium alkaloids were chromatographed as their free bases, acetates, and hydrochlorides. More variation was observed with the alkaloid salts than with the free bases. The results obtained with the latter using both the butanol-acetic acid solvent and the isoamyl alcohol-acetic acid solvent are reported in Table XXIII.

The spot patterns obtained with both solvents are similar, the general difference being the lower R_f values produced with isoamyl alcohol. That this lowering of R_f values is not proportionate throughout is seen by noting the B/A ratios given in the table. Morphine and codeine migrate at less than the average retarded rate. The reverse is true of narcotine and papaverine.

The values given for the butanol solvent represent averages of five determinations, three of which were run simultaneously, but in different chambers. The average variation between the R_f values of the alkaloids of the two non-simultaneous trials is 7.2 (0.9 to 15.8) percent. In the simultaneous trials the deviation from the mean never exceeded 3.2 percent and the average is only 1.9 percent. Variation is even less if one considers the relative position of the spots, that is, the relation of each

Table XXIII. Comparison of Solvents for Alkaloids

Alkaloid	Butanol- Acetic Acid Solvent	Isoamyl Alcohol Acetic Acid Solvent	B/A
	R _f (A)	R _f (B)	
Morphine	0.41	0.12	0.29
Narcotine	0.76	0.62	0.82
Papaverine	0.74	0.61	0.82
Thebaine	0.65	0.43	0.66
Codeine	0.48	0.20	0.42
Narceine	0.67	0.46	0.69
Heroin	0.61	0.36	0.59
Dionine	0.57	0.30	0.53
Apomorphine	0.65	0.33	0.51

alkaloid spot to all others on the same sheet. Such a consideration can be appreciated mathematically by comparing the R_f of each spot with the average R_f of all spots. Here, the deviation from the mean does not exceed 1.9 percent (average: 1.2%)

The values given for the isoamyl alcohol solvent were obtained from a single trial. Experience has shown that there is considerable variation in the R_f values obtained over a period of months. The following factors are known to influence R_f values: 1) the paper employed, 2) temperature, 3) quantity of material being chromatographed, 4) extraneous substances, 5) degree of saturation of the paper with water and solvent, 6) starting point with relation to solvent, 7) height of chromatograph, and 8) volume of starting spot. In these experiments temperature was the only factor not controlled. Indeed, it was found in many subsequent determinations where morphine, codeine, and heroin were employed as standards in toxicologic determinations that temperature had a definite influence on the R_f values obtained. The values reflected the effects of seasonal room temperature changes, higher temperatures giving higher R_f values. A summary of 92 trials employing the isoamyl solvent against morphine, codeine, and heroin gave respective R_f values of 0.151 (0.12 - 0.19), 0.243 (0.20 - 0.30), and 0.382 (0.31 - 0.47). These figures emphasize the necessity of simultaneously employing standards where unknown samples are being determined, especially when controlled temperature conditions do not prevail.

Two-dimensional chromatography: It is seen in Table XXIII that several of the R_f values fall within close range of each other. Compounds which group together in one-dimensional chromatographs can often be separated by two-dimensional chromatography. With this technic a spot is placed near the corner of a square of filter paper and chromatographed in the usual manner. After the solvent has run its course, the various compounds have aligned along one edge of the paper. When dry the paper is rotated 90 degrees and the spots rechromatographed using a different solvent. This new solvent flows through the horizontal row of spots and transports them vertically to another area of the paper commensurate with the R_f value of the compound in that solvent. The completed sheet shows the spots distributed in characteristic positions throughout the paper. By plotting the R_f values of one solvent as abscissae against the R_f values of another solvent as ordinates, it is possible to approximate the positions of spots as they would appear if these two solvents were used in a two-dimensional chromatograph (49). This has been done in Figure 3 where isoamyl alcohol-acetic acid solvent is used in the first phase of the two-dimensional scheme and isoamyl alcohol-ammonium hydroxide solvent* is used in the second phase. The points placed along the abscissa and ordinate show the expected positions of the alkaloids had they been developed in a one-dimensional system by the respective solvents. Experimental results closely

* This differs from the isoamyl-acetic acid solvent only in that concentrated ammonium hydroxide is substituted for glacial acetic acid.

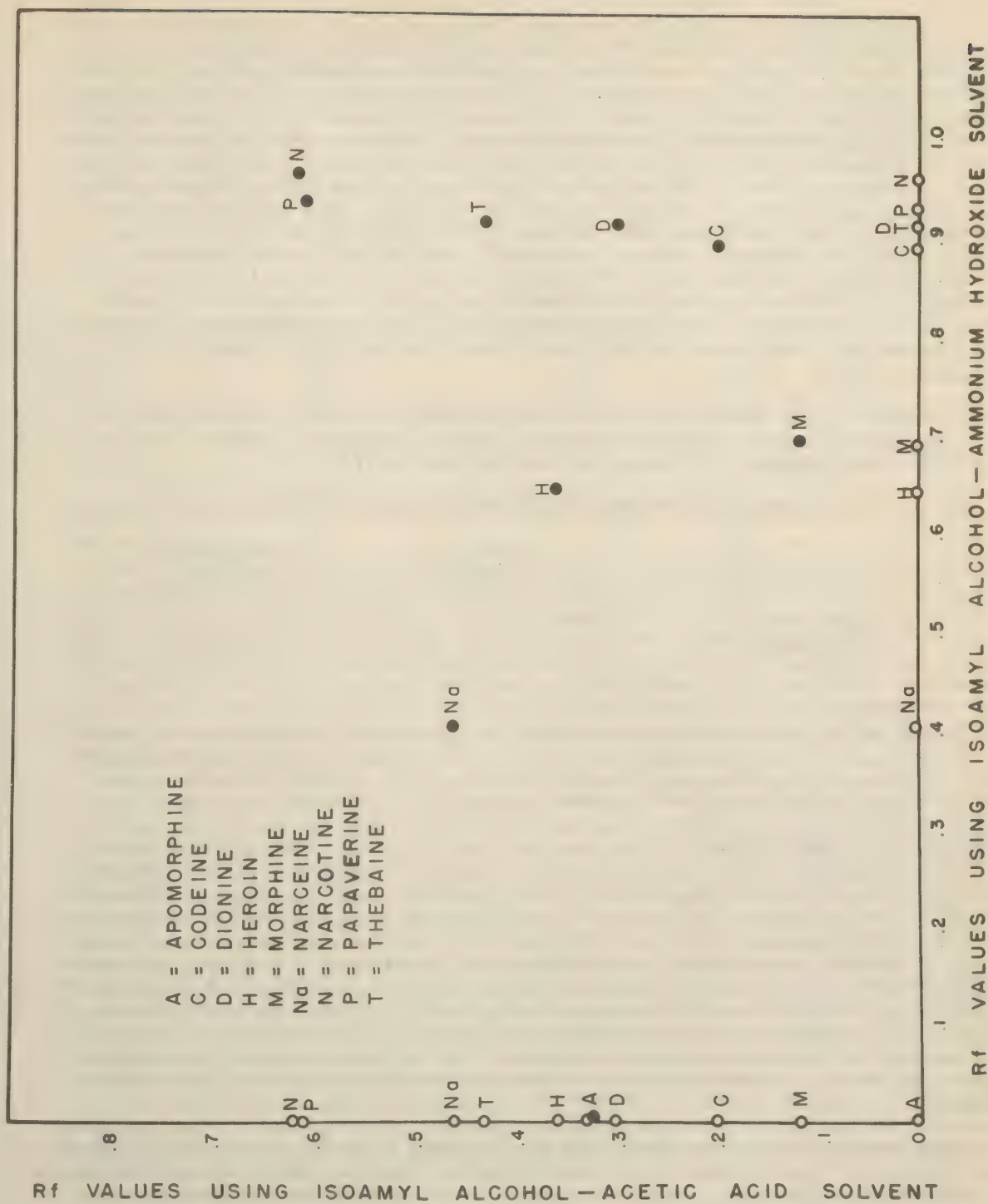


Figure 3. Diagrammatic Representation of Two-Dimensional Paper Chromatograph of Opium Alkaloids

approximate the calculated results shown diagrammatically. While this two-dimensional system is not ideal (e.g., papaverine and narcotine still remain unseparated), much of the crowding found in the one-dimensional schemes is eliminated.

Chromatography of alkaloids extracted from toxicologic specimens - The final dry extracts obtained from tissues by the Stas-Otto procedure or from blood or urine by a new procedure developed in this laboratory and outlined in the section for toxicology are transferred from 50 ml beakers to 13 x 100 mm lipless test tubes (Wassermann type) using 2 ml of boiling ethanol. The ethanol is removed by evaporation on a water bath. Three or four drops of ethanol are added to the tube and, while still in the water bath, the solution is drawn up and down a 0.1 ml Mohr pipette until a volume of 0.06 ml is reached. Four 0.005 aliquots of this are applied to the paper forming a single spot. Each aliquot is allowed to dry before making the next addition, thus keeping the area of the final spot at a minimum. The remaining two-thirds of the material is saved for chemical testing. Morphine, codeine, and heroin standards (free bases) serve as controls. An 0.5% solution of each is applied to a single point on the base line in a volume of 0.005 ml. The spots of the various extracts to be tested are located at 3 cm intervals on the base line, chromatographed in the described manner with the isocamyl alcohol-acetic acid solvent, and sprayed with the iodoplatinate reagent.

A comparison of the results obtained by chromatography and by chemical tests (Marquis, Frohde and Mecke) during the months of August, September, October, and November 1952, on a total of 221 cases is shown in Table XXIV.

Table XXIV. Comparison of Chemical and Chromatographic Detection of Alkaloids

	<u>Chemical Results</u>	<u>Chromatographic Results</u>
Negative	173	146
Questionable	20	30
Positive	28	45

All but a small number of the cases in Table XXIV involve blood and urine specimens. A good part of the discrepancy between the number of positive cases found by chemical and chromatographic methods results from the manner in which the urine was processed. Gross and Thompson (50) found that hydrolysis* of urine from morphinized dogs increased the yield of morphine four or five times. This is due to the release of morphine from a combined form not recovered by usual extraction procedures. In order to take advantage of this extra source of morphine, the hydrolysis procedure was incorporated in the extraction process. A comparison of hydrolyzed and unhydrolyzed urines soon verified the advantages of hydrolysis as was evidenced by the greatly increased sizes and intensities of the developed morphine spots. However, hydrolysis introduces a serious problem, that of increasing the impurities in the final extracts. These impurities are dark brown and mask the chemical color reactions to the extent that, even while greater amounts of morphine are present, a more positive conclusion could have been reached had the urine not been hydrolyzed. These impurities incur no disadvantages in the chromatographic process since they do not affect the progress of morphine migration and they rise to positions on the paper high above the morphine spot area.

The "questionable" cases of Table XXIV require some discussion. Almost all of those so classified from chemical tests were listed as positive chromatographically. On the other hand, those cases which were considered "questionable" chromatographically result from chromatographs where only very weak morphine spots were observed, where morphine spots were "off color", or where the R_f values were slightly different

* A suitable aliquot of urine was hydrolyzed with 10% by volume of concentrated hydrochloric acid in the autoclave by heating at 15 pounds pressure for 30 minutes.

from that expected for morphine. The very weak spots represent quantities of morphine less than 10 micrograms. Chemical confirmation of such minute amounts of morphine cannot be expected when it is remembered that the remaining two thirds of unchromatographed extract must be divided to make the several chemical tests.

The ideal chromatographic procedure would be specific for opium alkaloids and nothing else. The achievement of such an ideal is not likely because of the non-specificity of alkaloid isolation methods, R_f values, and spot detection reagents. That the procedure developed here is not ideal is evidenced by the appearance of occasional miscellaneous spots. The distribution of these extraneous spots from extracts of blood, urine, stomach contents, liver, and brain from 201 negative cases is given in Table XXV.

Table XXV. Distribution of Extraneous Chromatographic Spots

<u>Specimen</u>	<u>Total Cases</u>	<u>Cases Where Miscellaneous Spots Were Found</u>	<u>Percent of Cases Showing Miscellaneous Spots</u>
Blood	152	7	5
Urine*	86	18	21
Stomach Content	20	10	50
Liver	19	8	42
Brain	10	3	33

* Includes both hydrolyzed and unhydrolyzed samples

The high incidence of extraneous spots found in stomach contents extracts is an expected finding since a large variety of alkaloids are found in the plant foods commonly ingested. Blood and urine extracts showed the fewest spots, not only in the number of cases but in the number of spots per case. Undoubtedly some of these unidentified spots represent alkaloids of pharmacologic interest. Fortunately, few of these spots fall in the morphine area and, when they do, they show a violet coloration rather than the characteristic dark blue of morphine. These extraneous spots are quite often diffuse and leave trailing streaks in their wake. They are easily recognized by the experienced operator.

Of particular interest are those extraneous spots found in positive morphine extracts. In the sodium hydroxide-chloroform extract of most urine samples showing a strong morphine spot, a second spot is found in the codeine range. The persistent appearance of this spot in association with morphine suggests that it represents a metabolic end product of morphine, possibly codeine. The conversion of codeine to morphine in tissues has been demonstrated (51); that the reverse process might take place is not a fanciful suggestion. Quite often a third spot is found located between the codeine and heroin areas. A fourth spot is sometimes observed in the range of R_f 60 to R_f 70. This spot is most commonly seen in hydrolyzed specimens. Attempts at identification of these materials are being made with the hope that something may be learned about morphine metabolism. There is some evidence that addicts metabolize morphine in a different manner than novices (52, 53), and it is possible that the appearance of the spots associated with morphine in some cases and not in others may reflect the degree of addiction of the subject. An alternative explanation may be that because the urine samples are collected at different times after the drug is administered they represent various stages of morphine metabolism.

While heroine is undoubtedly the offending drug in most subjects, this alkaloid has never been observed on the chromatographs, thus supporting the view that heroine rapidly converted to morphine in the tissues.

These results show the validity of applying the paper partition chromatographic technic to toxicologic problems. They also point out the need for an improved method of purifying extracts.

Chromatographic purification of alkaloids extracted from toxicologic specimens: After establishing the presence of suspected opium alkaloids by chromatography using one-third of the final extracts, the remaining two-thirds of the material is purified chromatographically before attempting chemical identification (When very large spots are observed on the preliminary chromatograph, only half of the remaining material is used for purification). Instead of spraying this second chromatograph, small squares of paper are cut from areas corresponding to the spot areas found on the preliminary sheet. These areas are located by clipping out a strip of paper containing chromatographed standards (morphine, codeine, heroine, spraying this strip, and then replacing the strip to act as a guide. The areas to be extracted can be even more perfectly located by cutting a one millimeter strip up through the center of the chromatographic path, spraying this, and replacing it in the sheet. Areas on both sides of the color band on this narrow strip are removed for extraction. The squares of paper are rolled into a cylinder and placed in Wassermann-type test tubes. The paper is wetted with 2 drops of concentrated ammonium hydroxide and 2 ml of chloroform is added. The chloroform is boiled for a few seconds on a water bath and the chloroform extract transferred in 1 ml aliquots to a second test tube contained in the water bath, allowing the first portion of chloroform to evaporate before adding the second. Spraying of these small pieces of paper after extraction shows the alkaloid has been removed. Similar extractions employing ethanol proved unsuccessful; substances interfering with the chemical tests are extracted with ethanol. As a check against the possibility of one spot having "wandered" into the "lane" of an adjacent extract, the sheet from which the squares have been cut is sprayed with the iodoplatinate reagent. In the experience of this laboratory such migrations have not occurred, but it is felt that this precaution must be taken to obviate legal criticism.

The residue remaining after the chloroform is removed is colorless and of such minute quantity that only by close inspection can it be seen at all. For chemical testing, five drops of ethanol are added to the tube and the tube warmed briefly on a water bath. Aliquots of this are dried on a spot plate or microscope slide for testing. The resulting reactions are comparable to those seen with tests on pure alkaloid standards; background colors and charring are absent; the colors persist and show the classical transformations.

The chromatographic purification of alkaloids has been applied routinely in this laboratory only during the last three weeks of December, and, while there have not been sufficient cases to establish the absolute validity of the method, the results obtained thus far have been highly encouraging. Spots have been graded as to size and density so that a spot which is just discernible receives a rating of $\frac{1}{2}$ plus, a dense spot with dimensions of about $1\frac{1}{2} \times 2$ cm is classified as 3 plus, and other spots between $\frac{1}{2}$ and 5 are graded accordingly. It is estimated that a $\frac{1}{2}$ plus spot of morphine contains about 5 to 10 micrograms, and a 3 plus spot, 50 to 75 micrograms of the alkaloid. The chromatographically purified extracts were given a grade derived from the preliminary chromatograph, thus adjusting for the amount of extract used. Thirty-four urine extracts which showed preliminary chromatograph spots in the morphine area with grades ranging from $\frac{1}{2}$ to 5 plus were purified as described above. All but one of 21 purified residues with a 3 plus rating or greater gave positive chemical reactions. The single exception was obtained from an extract which showed a violet spot rather than the characteristic blue of morphine. Five $2\frac{1}{2}$ plus, three 2 plus, and two $1\frac{1}{2}$ plus urine residues were all positive. One 1 plus urine residue was positive, another negative. A single $\frac{1}{2}$ plus urine residue gave negative results. A purified extract from brain (1 plus) showed good positive chemical reactions. A codeine spot (3 plus) from a stomach contents extract was purified chromatographically and identified chemically. The advantages of paper purification become obvious when these figures are compared with those given for unpurified extracts in Table XXV.

More recently it has been found that morphine can be recovered directly from the sprayed spot. When the paper is sprayed lightly, only a small amount of the morphine combines with the platinate. This lightly sprayed spot is treated with ammonium hydroxide and extracted as described above. The residue thus obtained is indistinguishable

from that recovered from an unsprayed area. This modification of the procedure eliminates the preliminary chromatograph, and saves not only time, but working material. In addition, it enables perfect delineation of the spot to be extracted.

Paper chromatography has not been applied to quantitative analyses of the alkaloids in this laboratory, but the above studies indicate that the technic would adapt readily to such procedures.

DEPARTMENT OF BACTERIOLOGY

The mission of the Bacteriology Department during 1952 continued to include bacteriologic support of medical units in the Far East Command and the manufacture of diagnostic antigens and antisera for use by these units. Materials for intravenous therapy were processed and safety tested, and antibiotics were assayed for potency. Problems of major interest have included the anaerobic bacterial flora of war wounds, new methods in Clostridia isolation and identification, the bacteriology of enteric infections in Japan and Korea, the antigenic structure of Shigella, Leptospirosis, and the bacteriology of non-specific urethritis. The department is organized into 7 sections: Diagnostic and Sanitation Bacteriology, Anaerobic, Tuberculosis, Enteric, Biologics Products, Special Projects, and Service including media preparation, glassware processing, sterilization and animal caretaking.

A total of 134,728 routine examinations and procedures were carried out in processing 41,125 specimens. In addition, the production of diagnostic biologicals, tests for pyrogens, other safety tests and assays, and lyophilization of newly isolated cultures of scientific interest occupied a significant part of the effort of the staff. Although total procedures did not increase during 1952 as compared with 1951, the amount of media used increased by 1,000 liters, indicating the increased scope of investigative work pursued.

A summary of routine procedures carried out is presented in Table I.

Table I. Bacteriologic Routine Procedures, 1952

Smears:

Clinical specimens	4,015
Darkfields, including leptospira	528
Acid-fast bacilli	6,021

Cultures:

Blood	155
Urine	288
Transudates and exudates	111
Wounds and tissues (human)	1,367
Sputum	250
Ear, Nose, and Throat	1,527
Genito-Urinary, female	313
Male Urethral and Penile lesions	1,980
Stools and rectal swabs	2,106
Tuberculosis	9,041
Autopsy cultures (human)	322
Autopsy cultures (animal)	532
Food cultures	330
Organisms submitted for identification	4,882
Sterility tests	270
Leptospira cultures	206
Miscellaneous	763

Sanitary Bacteriology

Processed waters and ice	40,752
Raw waters	2,256
Dairy products	3,200
Food and miscellaneous	939

Animal Inoculations

Virulence and toxicity	50
Pyrogen tests	138
Clostridia pathogenicity determinations	1,247
Leptospira inoculations	59
Guinea pig inoculations for tuberculosis	1,818
Tuberculin tests	15,618

Diagnostic Antibody Determinations

Febrile agglutinins (Typhoid, paratyphoid, etc.)	5,568
Leptospira	1,847
Antistreptolysin	780

Diagnostic Procedures, other

Acrolein tests (Clostridia)	2,860
Antibiotic assay and blood level	101
Sensitivity of organisms to antibiotics	5,818
Animal inoculation	1,238
Animal autopsies	2,162
Streptolysin "O" production, cc.	900
Miscellaneous	6

Biologics Production

Autogenous vaccines	15
---------------------------	----

Diagnostic antigens, 5 cc. units:

Typhoid "O", "H"	388
Paratyphoid "A" and "B"	1,104
Proteus OX series	1,057
Pasteurella spp.	230
Brucella spp.	112

Diagnostic antisera produced, 1 cc units:

Shigella spp.	2,790
Leptospira, Salmonella, other	210
Single factor sera, Salmonella, and Shigella, cc..	511
Rabbit serum, sterile 10 cc. units	320
Other sera and culture material	184

Cultures lyophilized (new strains)	3,159
--	-------

Diagnostic antisera issued	896
Diagnostic antigens issued	2,354
Cultures issued	145

TOTAL PROCEDURES 132,444

Media Production

Plates	55,973
Slants	66,465
Tubes	156,950
Bulk (liters)	5,841.19

TOTAL LITERS 7,981.7

Average animal room census per month	765
--	-----

DIAGNOSTIC AND SANITATION BACTERIOLOGY

Routine work in this section included 67,951 procedures. New routine procedures introduced in 1952 included use of a blood-sodium azide-crystal violet transport medium for throat cultures, bioassay of thiamine using Lactobacillus fermentum, serologic typing of streptococci, and a modified Bondi disc method for rapid assessment of sensitivity of bacteria to antibiotics. Observations of unusual interest included the isolation of a motile streptococcus from spinal fluid, isolation of C. neoformans from a case of meningitis, and the demonstration of Bact. anitratum in three cases of meningitis. Special studies included observations on chancroid, non-specific urethritis, dermatophytosis, staphylococci, streptococci, and sanitation bacteriology.

CULTURAL STUDIES ON HEMOPHILUS DUCREYI: A high incidence of chancroid in the Tokyo area and the lack of any generally accepted technique for cultural demonstration of the causative agent dictated continued study of H. ducreyi. Diagnostic criteria comprise demonstration of characteristic gram-negative rods in direct smears and cultures from ulcers or buboes (1). Evaluation of direct smears from lesions is uncertain, due to the presence of non-specific gram-negative bacilli, and such smears in our hands have been wholly unsatisfactory. Media recommended for isolation of the organism have included clotted rabbit blood (2), blood agar plates (3), whole blood (4), defibrinated rabbit blood (5), and pancreatic casein digest agar. Excellent results in propagating both stock strains and morphologically typical organisms in cultures from lesions have been obtained in this laboratory by use of 50% tryptose phosphate-rabbit serum broth. This simple medium compared favorably with clotted rabbit blood in promoting formation of the characteristic parallel chains ("railroad-tracks") of gram negative streptobacilli pathognomic of H. ducreyi (6). Substitution of pooled Seitz-filtered human serum from serologic laboratory discards for expensive rabbit serum, resulted in a practical substrate which is available to any routine clinical laboratory.

The proportion of positive cultures observed is represented in Figure 1. No specific explanation for the changes in rate of positive cultures has been found, but it may be associated with a drop in incidence of this infection in the Tokyo area.

Use of Inhibiting Agents in Culture Media - Two strains of H. ducreyi from clotted rabbit blood cultures were isolated by subculture to blood agar plates containing sub-inhibitory amounts (0.05 mcg. per ml.) of chloramphenicol (7). After 150 additional cultures had been subcultured on such solid media with negative results other inhibiting agents were tried. Assuming that failure of the organism to grow on solid media may be due to the inhibiting action of concomitant flora, suppression of this growth might permit H. ducreyi colonies to appear. Crystal violet was tried in varying dilutions; it inhibited stock strains and fresh cultures of H. ducreyi at levels above 0.01%, while at this level concomitant flora survived. Next, a new Japanese antibiotic, "Luteomycin" (produced by strains of Streptomyces) was tested. It permitted growth of stock strains of H. ducreyi in concentrations up to 50 mcg per ml. This amount of "Luteomycin" inhibited the growth of concomitant flora from chancroid lesions. In view of this encouraging finding, 94 morphologically typical positive serum-broth cultures were plated on blood agar containing 40 mcg/ml and 60 mcg/ml of "Luteomycin" and incubated in the presence of 3% CO₂. The concentrations of blood in the medium were varied from 7.5% to 25%, and the agar base was enriched with fresh yeast extract. No isolates of H. ducreyi were recovered, although gram-positive concomitant flora was inhibited. Parallel tests with penicillin and chloramphenicol in suitable concentrations in the agar bases described were similarly unsuccessful.

When chloramphenicol, penicillin, and "Luteomycin" were each added to serum-broth medium in concentrations tolerated by stock strains of H. ducreyi, a marked reduction in the gram-positive flora observed in routine cultures was achieved, while H. ducreyi survived unaltered. At the time of reporting the organism has not yet been isolated in pure culture from these preparations. However, its recognition is greatly facilitated by reduction of extraneous growth, and to this extent, the technique has been a success.

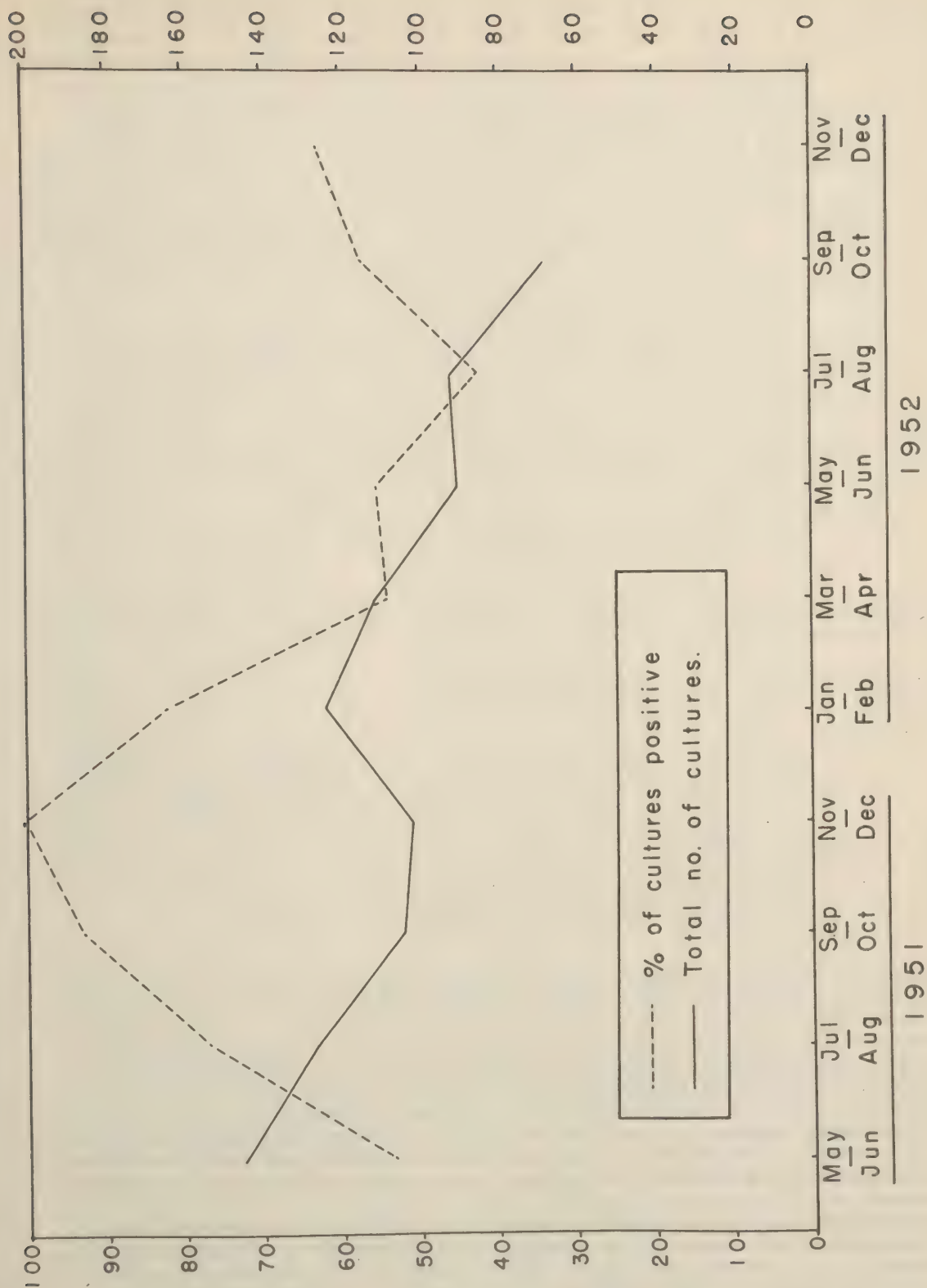


Figure 1. Seasonal Variation of *H. ducreyi* Cultures, 1951-1952

Perpetuation of Strains of *H. ducreyi* in Mixed Culture by Serial Transfer - The typical morphology of *H. ducreyi* in mixed culture (7) is relatively transient, and no report of its maintenance in serial cultures has been found. During experiments with antibiotics in broth cultures, it was found that typical strains could be propagated for at least 2 months by serial transfer at 48-hour intervals in serum-broth. A series of 20 cultures have been thus maintained and are now in their 34th subculture. After one or two transfers in serum broth containing penicillin, strains could be perpetuated in serum broth alone. Chain formation was regularly demonstrable in these cultures at 18-24 hours. When such chaining began to decrease on transfer, passage through penicillin-serum broth enhanced the chain development. It was assumed that such acclimation would permit establishment of growth on solid medium, but subcultures have thus far failed to yield isolates. Such failures may be due either to bacterial antagonism or to a symbiotic relationship with organisms in the mixed flora. Further enrichment of the broth cultures appeared only to enhance the amount of contaminating growth. Decreased agar content, and the use of surface-active agents in agar in both cases increased the spreading tendency of the contaminants present.

Comparison of Cultures from Cleansed and Uncleansed Lesions - It has been suggested that cleansing the chancroid ulcer prior to culture enhances recovery of the causative agent. A comparative study of this factor was undertaken on 420 cases, over an 8 month period. Cultures were taken with a dry swab from the gently dried lesion, after which the ulcer was gently but thoroughly scrubbed with a saline-soaked sterile sponge, rinsed well, and dried with sterile gauze. A second culture from the clean surface was then taken. All cultures were examined microscopically at 24, 48, 72, and 96 hours. Comparative results are summarized in Table II.

Table II. Comparison of 420 Cultures of Cleansed vs Uncleansed Chancroidal Lesions

<u>Incubation Time</u>	<u>Total Positives*</u>	<u>Positive C, U</u>	<u>Positive U Only</u>	<u>Positive C Only</u>	<u>Negative</u>
24 hours	39	17	18	4	-
48 hours	98	49	40	9	203
72 hours	22	14	6	2	49
96 hours	1	-	1	-	8
	160	80 (50%)	65 (90.6%)	15 (9.4%)	260

C, U = positive on both cleansed and uncleaned lesions
 U = positive on uncleaned lesions only
 C = positive on cleansed lesions only
 * = Organism resembling *H. ducreyi* seen in culture

Of 160 positive broth cultures, 80 were positive both before and after cleansing the lesions, 65 only before the lesions were cleansed, and 15 only after cleansing the lesions. It was apparent that cleansing the chancroid lesion prior to initial broth culture reduced the effectiveness of the culture. At least 48 hours incubation was usually required to demonstrate a positive or conform a negative culture in either case. Contaminating flora was comparable in both types of preparations. Positive *H. ducreyi* cultures were invariably accompanied by gram-positive cocci. The uncleaned preparations frequently showed gram-negative organisms morphologically dissimilar to *H. ducreyi*. The amount of growth of these organisms was significantly less from cleansed lesions than from uncleaned lesions after 48 hours of incubation.

Of 160 positive cultures 145 (91%) were demonstrated by culture of uncleaned lesions. Although an additional 9% were subsequently recovered by culture of the cleansed lesion, it is probable that had a second culture of the uncleaned lesion been made, the total recovery rate would have been as high. It was concluded that culture of the uncleaned lesion in 50% serum-tryptose broth constitutes at present the optimal technique for presumptive demonstration of *H. ducreyi*.

BACTERIAL FLORA OF NON-SPECIFIC URETHRITIS: A continued increase in the incidence of non-specific urethritis (i.e. urethritis not ascribable to demonstrable gonococcal infection) among troops in Japan occasioned more intensive study during 1952. Available references emphasized chiefly clinical manifestations, and few systematic bacteriologic studies of this condition have been reported. Hypothetical causative agents have included viruses (8), pleuropneumonia-like organisms (9), and mechanical causes (10).

Specimens of urethral exudate were collected by swab and inoculated directly to blood agar and chocolate agar plates which were incubated aerobically and under 3% CO₂ atmosphere in a candle jar. Serum-broth tubes were inoculated in a series of 50 cases for supplemental study of enrichment broth cultures. In cases in which gonococci were not recovered, the predominant aerobic flora was determined, and such cases were included in the non-specific urethritis group. With few exceptions, these patients had already been diagnosed as negative for gonorrhea.

The predominant strains of bacteria observed in 573 cases have been summarized on a monthly basis to demonstrate the consistent pattern of incidence of predominant organisms. Table III shows the distribution of the predominant species.

Table III. Predominant Organisms, Non-specific Urethritis, 1952

	<u>J</u>	<u>F</u>	<u>M</u>	<u>A</u>	<u>M</u>	<u>J</u>	<u>J</u>	<u>A</u>	<u>S</u>	<u>O</u>	<u>N</u>	<u>D</u>	<u>Total</u>
<u>Streptococcus, alpha-hemolytic</u>	4	7	5	2	1	3	4	0	4	4	6	7	47
<u>Streptococcus, beta-hemolytic</u>	3	7	8	1	0	0	1	0	0	9	8	7	44
<u>Streptococcus, non-hemolytic</u>	3	5	4	0	0	2	1	3	3	4	10	8	43
<u>Staphylococcus, H/S/M/C</u>	10	1	3	2	1	2	4	0	4	3	7	2	39
<u>Staphylococcus, H/S/M/C-</u>	9	5	8	3	0	3	5	9	14	20	31	24	131
<u>Staphylococcus, H/S/M-C-</u>	19	15	26	12	2	3	6	13	8	13	42	44	203
<u>Staphylococcus, H-S/M/C-</u>	3	1	0	0	0	0	0	0	0	0	0	0	4
<u>Staphylococcus, H-S/M-C-</u>	6	7	3	2	0	1	1	0	2	2	2	0	26
<u>Staphylococcus, H-S-M-C-</u>	1	0	1	0	0	0	0	0	0	0	1	2	5
<u>Hemophilus influenzae</u>	1	1	1	0	0	1	0	0	1	3	6	3	17
<u>Coliforms*</u>	0	0	2	0	2	1	0	2	1	2	1	5	16
<u>Proteus spp.</u>	0	1	1	0	0	0	0	0	0	0	0	0	2
<u>Corynebacterium pyogenes</u>	10	3	1	0	0	0	1	1	0	1	1	1	19
<u>Corynebacterium xerosis</u>	1	3	2	1	0	0	0	2	1	0	8	11	29
<u>Corynebacterium diphtheriae</u>	0	0	1	0	0	0	0	0	0	0	0	0	1
<u>Corynebacterium hoffmanni</u>	0	1	6	0	0	0	1	0	0	3	8	6	25
<u>Corynebacterium spp.</u>	0	0	0	0	1	0	0	0	0	1	0	2	4
<u>Candida albicans</u>	0	1	0	0	0	0	0	0	0	0	0	0	1
<u>No organism isolated</u>	4	5	2	0	0	0	2	1	3	2	1	0	20
<u>Total</u>	<u>74</u>	<u>63</u>	<u>74</u>	<u>23</u>	<u>7</u>	<u>16</u>	<u>26</u>	<u>31</u>	<u>41</u>	<u>67</u>	<u>132</u>	<u>122</u>	<u>676</u>

* Includes Escherichia, Aerobacter, Alkaligenes, and Paracolon

An obvious feature of this tabulation is the predominance of two groups of staphylococci. Both varieties were hemolytic, salt-resistant, and coagulase-negative, but the strains varied in their ability to ferment mannitol. A total of 234 cases yielded strains of these two patterns. Two hundred one, or 61% of these strains were resistant to 1 unit of penicillin in an absorbent disc on a blood agar plate.

It is premature to imply that the staphylococci described bear a causal relationship to non-specific urethritis. However, clinicians treating these patients came to regard the finding as significant and consistent with the clinical state of the patient. Further study of these strains is to be carried on.

No previous mention of H. influenzae in urethral exudates has been reported. Although the number of cases is not large, this organism is definitely exotic in this environment, and calls for further investigation.

Corynebacterium strains of 5 species totalled 78 strains. These were sought with particular emphasis in view of references in the literature to a possible etiologic relationship. The minute hemolytic strains, classed as C. pyogenes, were difficult to recognize.

The absence of Enterobacteriaceae indicated the limited range of the flora in non-specific urethritis in Japan. The uniform distribution of beta, alpha, and non-hemolytic streptococci rendered their presence of less potential significance, although the total of 134 strains made them the second largest generic group of organisms observed.

CULTURAL PATTERNS AND ANTIBIOTIC SENSITIVITY OF STAPHYLOCOCCI: Four reactions which are customarily associated with evaluation of pathogenicity of staphylococci are hemolysis, salt tolerance (7.5% NaCl in agar), mannite fermentation and coagulase formation. During 1952 these factors were observed on a series of 858 strains obtained from cultures of human urine, spinal fluid, pus, eye, ear, nose, throat, urethra, cervix, and blood. Wide variations in these cultural characteristics has often been described as representing random distribution. However, a very limited range of reaction patterns appeared in these cultures (Table IV).

Table IV. Reaction Pattern of 858 Strains of Staphylococci Isolated During 1952

<u>Cultural Pattern</u>	<u>No. of Strains</u>	<u>% of Total</u>
H ⁺ , S ⁺ , M ⁺ , C ⁺ (1)	286	33.3%
H ⁺ , S ⁺ , M ⁺ , C-	223	25.9%
H ⁺ , S ⁺ , M-, C-	263	30.6%
H ⁺ , S-, M-, C-	4	0.46%
H-, S ⁺ , M-, C-	52	6.06%
H-, S ⁺ , M ⁺ , C ⁺	16	1.8%
H-, S ⁺ , M ⁺ , C-	10	1.1%
H-, S-, M-, C-	4*	0.46%

(1) H⁺ = Hemolytic S⁺ = Salt-resistant M⁺ = Mannite fermented
 C⁺ = Coagulase formed H- = Non-hemolytic S- = Salt sensitive
 M- = Mannite not fermented C- = Coagulase not formed

* One strain toxigenic

Of the 16 possible reaction combinations, only 8 were found, and 89.8% of all strains fell in three reaction patterns. All were hemolytic and salt resistant, but varied in coagulase and mannite fermentation. Inability to split mannite was shown to be correlated with absence of coagulase formation; none of 323 mannite-negative strains was coagulase-positive. Therefore, the coagulase reaction need be performed only on mannite-positive strains. No significant seasonal variation in distribution of strains with regard to reaction patterns was noted.

A striking instance of toxin formation in a strain which was inactive in vitro was found during investigation of an outbreak of food poisoning. The strain was H-, S-, M-, C-, but produced an active persistent enterotoxin. This is, in all probability, very uncommon. It emphasizes the lack of final validity in biochemical patterns of staphylococci and the necessity for animal test of all suspected enterotoxic strains.

Correlation of sensitivity to penicillin, aureomycin, terramycin, and streptomycin of 145 strains of staphylococci was studied. Sterile absorbent discs (Whatman #2 filter paper) 12 mm. in diameter were placed on seeded blood agar plates and moistened with 0.03 ml. of antibiotic solution from a tuberculin syringe. These antibiotic solutions were adjusted to contain 1 unit of penicillin, 2.5 mcg. of aureomycin, 2.5 mcg. of terramycin, or 12.5 mcg. of streptomycin, respectively, per each 0.03 ml. A growth-free inhibition zone was interpreted as indicating sensitivity; absence of an

inhibition zone indicated resistance to the pertinent antibiotic. Distribution of sensitivity of 145 strains is summarized in Table V. Strains are presented with reference to penicillin sensitivity as the primary criterion.

Table V. Sensitivity of 145 Strains of Staphylococci to Penicillin, Aureomycin, Terramycin, and Streptomycin

Sensitive to Penicillin Antibiotic*					Resistant to Penicillin Antibiotic*				
Pen. 1 u.	Aureo. 2.5 mcg	Ter. 2.5 mcg	Strept. 12.5 mcg	No. Strains	Pen. 1 u.	Aureo. 2.5 mcg	Ter. 2.5 mcg	Strept. 12.5 mcg	No. Strains
S	S	S	S	44	R	S	S	S	41
S	R	R	S	8	R	R	R	S	11
S	S	S	R	3	R	S	S	R	8
S	S	R	R	2	R	S	R	R	3
S	S	R	S	1	R	R	S	R	2
S	R	S	S	1	R	S	R	S	1
S	R	S	R	0	R	R	S	S	1
S	R	R	R	0	R	R	R	R	19
Total				59	Total				86

*S: Sensitive; R: resistant

In terms of comparative sensitivity, 44, or 74.5%, of the penicillin-sensitive strains were also sensitive to the other three antibiotics. Conversely, of the penicillin-resistant strains, 19, or 22%, were resistant to all other antibiotics in the concentrations employed. With due regard for the obvious fact that these relationships are based on empirically selected concentrations of antibiotic, it appears that in 75% of the cases, a strain sensitive to penicillin would be sensitive to all other antibiotics. In the case of penicillin-resistant strains, 41, or 47.6%, were sensitive to all other antibiotics. The single antibiotic most likely to be effective against these was streptomycin, which inhibited 62.7% of the strains. It is of significance that while 22% of penicillin-resistant strains were also resistant to all other antibiotics tested, there were no instances of a penicillin-sensitive strain being resistant to all of these antibiotics. The nature of this difference is not within the scope of this study, although several explanations involving fundamental biological mechanisms have been proposed by Bondi (12), Barber (13), and Eagle (14).

Considering the group tested as a whole, 68.9% of strains were sensitive to terramycin, 71.0% to aureomycin, and 74.5% to streptomycin.

The relationship of cultural pattern to antibiotic sensitivity was studied. The 19 strains resistant to all 4 antibiotics and the 44 strains sensitive to all 4 were compared by reactions. Table VI shows the lack of general correlation between reaction and sensitivity. Among the antibiotic-sensitive strains the mannite-positive, coagulase-negative pattern is the least likely of the major reaction patterns to occur.

FUNGI IN CULTURES OF DERMATOPHYTOSES FROM AMERICANS IN JAPAN: The predominant fungus infections studied in this laboratory have been dermatophytoses. The distribution of dermatophytes and the relative incidence of these species and of common contaminants is of epidemiologic interest. Table VII gives the predominant pathogenic species and sources encountered during 1952.

In addition to the 214 strains listed as pathogens, 169 strains of common contaminants were identified from skin sources, including 23 strains of Aspergillus,

Table VI. Reaction Patterns of Staphylococci Resistant to and Sensitive to Antibiotics

Reaction Pattern	Behavior to Antibiotics	No. of Strains	% of all Resistant Strains
H ⁺ , S ⁺ , M ⁺ , C ⁺	Resistant	9	47.4%
H ⁺ , S ⁺ , M ⁺ , C ⁻	Resistant	6	31.6%
H ⁺ , S ⁺ , M ⁻ , C ⁻	Resistant	4	21.0%
			% of all Sensitive Strains
H ⁺ , S ⁺ , M ⁺ , C ⁺	Sensitive	18	40.9%
H ⁺ , S ⁺ , M ⁺ , C ⁻	Sensitive	2	4.5%
H ⁺ , S ⁺ , M ⁻ , C ⁻	Sensitive	15	34.1%
H ⁺ , S ⁻ , M ⁻ , C ⁻	Sensitive	1	2.3%
H ⁻ , S ⁺ , M ⁻ , C ⁻	Sensitive	8	18.2%

Table VII. Pathogenic Fungi Recovered During 1952

Source	Scalp, face, neck	Axilla and hand	Inguinal and foot	Other	Source Unknown	Total
<u>C. albicans</u>	4	13	76	16	22	131
<u>Trichophyton mentagraphytes</u>	0	1	34	12	0	47
<u>Trichophyton rubrum</u>	3	1	19	1	0	24
<u>Trichophyton sulfureum</u>	0	0	1	4	0	5
<u>Trichophyton beigelli</u>	0	0	0	1	0	1
<u>Microsporium gypseum</u>	1	3	0	0	0	4
<u>Epidermophyton floccosum</u>	0	0	2	0	0	2
Total	8	18	132	34	22	214

45 of Penicillium, and 47 of Candida (stellatoidea and kruzei). The remaining 54 strains were distributed among 19 genera of common saprophytes.

A marked increase in the number of isolations of Candida albicans during the last 6 months of 1952 occurred (Table VIII). No explanation for this peculiar epidemiologic phenomenon has been found. The majority of cases occurred among troops billeted in a 3,000 bed dormitory, and ample opportunity for spread of infection was present.

A problem in species differentiation arose during the year with regard to Trichophyton rubrum and T. mentagraphytes. Infections with these two species vary markedly in severity, and accurate identification of strains is of value in prognosis and treatment (15). Although the classic descriptions of these species (16) imply that no difficulties are inherent in their recognition, the strains recovered here included an intermediate group that presented a source of confusion. T. mentagraphytes customarily forms a white powdery colony with abundant formation of microconidia. In addition, structures including coils, nodular bodies, and macroconidia (fuseaux) are frequently present. Pigment is formed slowly to present a light buff to rose-tan color, and is much less intense and less widely diffused than that formed by T. rubrum. Cultures

Table VIII. Incidence of Candida albicans Isolated in 1952

	<u>Candida albicans</u>
January-March	2
April-June	11
July-September	60
October-December	57

of I. rubrum are cottony due to the abundant mycelia; microconidia are scarce, and coils, nodular bodies and macroconidia are rare. Pigment formation is intense and the red-purple pigment frequently diffuses into the marginal hyphae.

An intermediate form, occurring in patients in Tokyo, has been recognized. Moderate amounts of microconidia may be formed, and a delayed but ultimately intense pigment is formed. Since neither of these characteristics is stable, it is important that identification be accomplished on the initial isolate when possible. The relationship of the cultural patterns observed is shown in Table IX.

Table IX. Differentiating Criteria of Predominating Trichophyton Spp., Tokyo, 1952

	<u>Microconidia</u>	<u>Nodular Bodies, Macroconidia, Coils, etc.</u>	<u>Pigment</u>
<u>T. mentagraphytes</u>	+++	++	+
Intermediate form	++	+	+++
<u>T. rubrum</u>	+	+	++++

STREPTOCOCCAL PHARYNGITIS AND THE STREPTOCOCCI OF POWDERED MILK: In March 1952, there occurred an explosive outbreak of 163 cases of streptococcal pharyngitis, on the USS Breckenridge, a military transport. Twenty-six strains of hemolytic streptococci were recovered from 64 patients. Mass penicillin treatment arrested the outbreak promptly. Cans of powdered milk from shipboard stocks were submitted for culture in an attempt to locate the source of the outbreak. Eleven of 12 previously unopened cans yielded beta hemolytic streptococci. It was assumed that these represented the probable source of the infection. Serologic classification was not immediately available, and differentiation of the milk and throat strains by biochemical reactions was attempted. A simple and generally applicable classification technique was applied, based on the pattern described by Sherman (17). Comparative results of reactions are summarized in Table X. Serologic grouping results are appended, although differentiation of the significant groups did not require this data.

Strains of a single group of streptococci frequently show diverse fermentative patterns (18). The throat strains were readily differentiated from milk powder strains, since the latter tolerated 6.5% NaCl and 40% bile, survived 60°C. for 30 minutes, and fermented sorbitol and mannitol. These strains belonged to group D. Those throat strains which were group A were negative in each of these tests. Of 2 group C strains, one grew in 0.1% methylene blue, while the other did not. Group G strains gave more diverse reactions than those of group A; however, they could with 3 exceptions be differentiated from group D strains by their failure to ferment sorbitol and by sensitivity to heat. The streptococci from milk powder were relatively homogeneous in biochemical reactions; their bile tolerance, heat resistance, and fermentation of sorbitol were key criteria in differentiating them from human strains of groups A and G.

The potential error of confusing the group D streptococci frequently present in powdered milk with human pathogens should be avoided. The cultural reactions pointed

Table X. Reactions of Human and Milk Strains of Streptococci Recovered in Shipboard Outbreak of Pharyngitis

Reaction	No. of Strains: Throat							No. of Strains Milk Powder		
	10	2	3	4	3	3	1	7	2	2
Growth in 0.1% Meth. Blue	-	+	+	-	-	+	+	+	+	+
Milk Coagulated	-	-	-	+	-	+	-	+	+	+
Milk Digested	-	-	-	-	-	-	-	-	+	-
Growth in 6.5% NaCl	-	-	-	-	-	+	+	+	+	+
Growth in 40% Bile	-	-	-	-	-	+	+	+	+	+
Gelatin liquef.	-	-	-	-	-	-	-	-	+	+
Survival 60°C. 30 min.	-	-	-	-	-	+	-	+	+	+
Final pH in Glucose	5.12-6.7	5.8	4.8-5.92	4.8-5.02	4.75	4.1	4.9	4.13	4.11	4.15
Sorbitol	-	-	-	-	-	+	-	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	-	+	-	-	+	+
Mannitol	-	-	-	-	-	+	-	+	+	+
Lactose	+	-	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	+	+
Inulin	+	-	+	-	-	-	-	-	-	-
Raffinose	-	+	-	-	-	-	-	-	+	-
Starch	+	-	+	-	-	-	+	+	+	-
Lancefield Group	A	C	G	G	G	G	G	D	D	D

± = variable; predominantly positive

∓ = variable; predominantly negative

out furnish a simple means of accomplishing this distinction. The significance of the group G streptococci in this outbreak is not known, but such "higher" types may at times be incriminated in human infections.

WATER AND DAIRY BACTERIOLOGY IN JAPAN: A large part of the routine work of the Bacteriology Department consists of the examination of water, ice, and dairy products. The average annual pollution rate of water and milk gives a basis for evaluating the value of plate counts.

The correlation between plate count and coliform content of water supplies may be of significance although it is not widely employed in the United States. From the operational standpoint, circumstances may occur in which completed coliform tests would not be feasible although total plate counts could be carried out. The relationship of these two values was studied on a statistically significant number of samples, and is summarized in Table XI.

Table XI. Coliform Content and 48 Hour Plate Count on Finished and Raw Waters in the Tokyo Area, 1952

<u>Water Sample</u>	<u>No. Samples</u>	<u>Coliform Test</u>		<u>Plate Count</u>	
		<u>Positive</u>	<u>% of Total</u>	<u>Over 200/ml.</u>	<u>% of Total</u>
Chlorinated:					
Drinking tap	17,952	278	1.6	938	5.2
Filter Plant	701	4	0.6	4	0.6
Reservoir	322	4	1.2	1	0.3
Ice	509	58	11.4	28	5.5
Well	799	9	1.1	21	3.0
Swimming Pool	364	28	7.7	92	25.2
Unchlorinated:					
Filter Supply	76	76	100.0	10	13.1
Lake and Beach	52	33	63.4	20	38.4
Stream	12	2	16.6	2	16.6

Coliform content and plate count showed a variable correlation in samples from different sources. For tap water the number of positive (over 200 per ml.) plate counts was 3 times that of positive coliform tests, probably due to growth during transport of un-iced specimens. However, the percentage relationship justifies plate count as a usable emergency technique for evaluation of water supplies. Manufactured ice offers less possibility for proliferation of organisms during storage and transport, and showed positive plate counts in half the total of positive coliform tests. Swimming pool water is inevitably contaminated with saprophytic flora which result in a proportionately high count. During 1952 an extensive study of ice supply in the Far East was carried out. This was occasioned by complaints that the ice supplied to Korea from Japan was non-potable. Careful bacteriologic examination indicated that most of the contamination of ice occurred in handling the finished product rather than its having been prepared from polluted water.

Unchlorinated samples showed fewer positive plate counts than coliform positives. Lake and ground water were highly contaminated and correlation of plate and coliform count was not evident.

Dairy products yielded coliform bacteria in an annual average of 15.3% of samples. Ice cream was the most serious offender, averaging 21.4% positive samples. Since all these products are prepared from dehydrated powders, which yield few coliform bacteria when cultured, the source of coliforms lies in the processing and handling of the products. The relationships of coliform content and plate count level in dairy products is shown in Table XII.

Table XII. Coliform Content and Standard Plate Count of Dairy Products

Sample	Number	Coliform		Average Plate Count	
		Positive	% of Total	Coliform Positive	Coliform Negative
Milk, plain	489	69	14.1	2800	240
Milk, chocolate	299	44	14.7	3800	1900
Buttermilk	232	13	5.6	50	40
Cream	88	13	14.7	1100	170
Ice Cream	579	124	21.4	2775	525

A positive correlation between coliform content and high average plate count was observed. Total count was 11-fold higher in coliform-positive milk than in coliform-free milk. The same ratio was 6-fold for cream, and 5-fold for ice cream. Coliform-free chocolate milk had an average plate count of half that noted in the coliform-

positive samples. Buttermilk, due to acid pH, is low in total count and in coliforms. A plate count could furnish an adequate presumptive screening for milk if necessary.

CLOSTRIDIAL STUDIES: During 1952, studies have included continuing observations on Clostridial flora in infected wounds under treatment in Japan, the bacterial flora of amputated extremities, a detailed study of the recently wounded (in collaboration with the Surgical Research Team) and new or improved technical procedures for isolation and identification of anaerobes.

Anaerobic Studies of Patients Evacuated to Japan - During 1950 and 1951, new techniques for isolation and identification, and determination of species incidence of Clostridia in wounds and amputation specimens were reported (7). These cultures were taken 5 or more days after injury, and on amputation the specimens were usually obtained at an average of 2 weeks after wounding. Generalized gas gangrene has been rare in the Korean War, but the possibility that Clostridia interfere with healing and contribute to shock has been considered. (18)

Clostridia in Amputated Extremities - In collaboration with the Pathology Department, cultures were made on extremities amputated after evacuation to Japan, beginning in September, 1952. Tissue blocks were collected from the site of amputation and at various intervals distally to determine the distribution of Clostridia in devitalized and non-devitalized tissues. Cross-contamination of specimens was avoided by using fresh sterile instruments and by sterilizing skin surfaces for each tissue sample. The aerobic flora was determined in most cases. Amputated extremities from 41 patients (109 specimens with 2 or more tissue blocks from 35 extremities) were cultured. One hundred and nine strains, comprising 16 species of Clostridia, were recovered from 33 (80.5%) of the extremities and from 67 (61.5%) of the specimens. The average number of strains per amputated extremity was 2.66, and if only cases yielding Clostridia are considered, was 3.33 strains per case. An average of one strain per specimen was recovered. Forty-four percent of the extremities and 16.5% of all specimens cultured yielded Cl. perfringens. A similar series of specimens observed in 1951 yielded 13.9% of Cl. perfringens.

Clostridia in Wounds First Cultured After Evacuation to Japan - The flora of 73 infected war wounds (83 specimens) was observed in order to compare this with the flora recovered from amputated extremities. Clostridia were recovered from 56 specimens (67.4%). In all, there were 90 strains recovered comprising 19 species. Cl. perfringens was found in 18.8% of wounds harboring anaerobes. This is in contrast to 1951, when 12% of the patients showed Cl. perfringens. A comparison of the total Clostridial flora in wounds and in amputation specimens is shown in Table XIII.

Table XIII. Clostridia Recovered From Wounds and From Amputation Specimens

	War Wounds	%	Amputation Specimens	%	Total	%
Cases	73		41		114	
Clostridia present	46	63.0	33	80.5	79	69.2
Total specimens	83		109		192	
Total specimens positive for Clostridia	56	67.4	67	61.5	123	64.0
Total Strains recovered	90		109		199	
Total species	19		16		22	
Ave. No. Strains per positive case	1.9		3.3		2.5	
Ave. No. Strains per positive specimen	1.6		1.0		1.03	

The preparation of cases harboring *Clostridia* was similar for wounds and for amputations. The average number of strains recovered was higher for amputated extremities than in soft tissue wounds. Conversely, the number of strains per specimen was higher in the wound than in the amputation. The similarities between the two types of cases are closer than had been found in previous comparable observations. The species recovered from the two sources are presented in Table XIV. *Cl. sporogenes*, *Cl. novyi*, and *Cl. paraputrificum* showed the principal variation in species incidence between wound and amputation, while *Cl. paraputrificum* was found only in the soft tissue wounds. In contrast to findings in 1951 (7), when *Cl. perfringens* was present 3 times as often in wound cultures as in amputated extremities, *Cl. perfringens* occurred with almost equal frequency in both during the studies in 1952. The majority of strains present were of species non-toxic for guinea pigs. It is possible that such strains cause tissue damage, toxic effects, and retardation of healing in humans.

Five species, including *perfringens* and *novyi* in the toxigenic group, and *sporogenes*, *multifermentans*, and *bifermentans* among the so-called non-pathogens, accounted for 81.6% of the amputation strains but only 60% of the strains from wounds. Twelve of the 22 species recovered occurred in both series; 3 were found only in amputation specimens, while 7 occurred only in tissue specimens. An interesting observation was the recovery of 22 strains of *Cl. multifermentans* since its occurrence in wounds has been considered unlikely (20). Two other unusual observations were the isolation of *Cl. fesceri* and *Cl. pasteurianum*. It is believed this is the first report of *Cl. pasteurianum* in human tissue. *Cl. fesceri* was first isolated in humans in 1951 at this laboratory.

Table XIV. Species of *Clostridia* Recovered from Wound Cultures and Amputation Specimens

Species	Cultures from Wounds	% of Total Positive Patients	Cultures from Amputation Specimens	% of Total Pos. Cases	Grand Total	% Pos.
<i>sporogenes</i>	19	21.1	38	34.8	57	28.6
<i>perfringens</i>	17	18.8	18	16.5	35	17.6
<i>multifermentans</i>	8	8.8	14	12.8	22	11.0
<i>bifermentans</i>	7	7.7	10	9.1	17	8.6
<i>novyi</i>	3	3.3	9	8.2	12	6.0
<i>lentoputrescens</i>	4	4.4	4	3.6	8	4.1
<i>cochlearium</i>	4	4.4	3	2.6	7	3.5
<i>histolyticum</i>	3	3.3	2	1.8	5	2.5
<i>carnis</i>	1	1.1	2	1.8	3	1.5
<i>aerofaecidum</i>	1	1.1	2	1.8	3	1.5
<i>fesceri</i>	0		1	0.9	1	0.5
<i>tetani</i>	0		1	0.9	1	0.5
<i>pasteurianum</i>	0		1	0.9	1	0.5
<i>tertium</i>	3	3.3	1	0.9	4	2.0
<i>tetanomorphum</i>	1	1.1	1	0.9	2	1.0
<i>sordelli</i>	1	1.1	2	1.8	3	1.5
<i>septicum</i>	3	3.3	0		3	1.5
<i>paraputrificum</i>	8	8.8	0		8	4.1
<i>filiforme</i>	2	2.2	0		2	1.0
<i>sphenoides</i>	2	2.2	0		2	1.0
<i>butyricum</i>	1	1.1	0		1	0.5
<i>difficile</i>	1	1.1	0		1	0.5
Unidentified	1	1.1	0		1	0.5
Total	90		109		199	

It will be noted that only one strain in Table XIV is listed as "unidentified". This is in sharp contrast with earlier reports, where the unidentified strains were as high as 35% (21), (22), (23). Our success in anaerobic identification is due principally to elimination of anaerobic streptococci from cultures, the techniques for which are described later in this section. These organisms frequently confuse the biochemical reaction pattern, thus preventing recognition of a Clostridium strain.

The overall picture of Clostridia in infected wounds and amputations does not indicate completely the ecologic relationships observed in multiple samplings from amputated extremities. These relationships are presented in Table XV. The amputations followed leg wounds with extensive tissue damage, frequently with compound comminuted fractures. There was no consistent pattern for the topical distribution of Clostridia. In two instances tissue at the line of amputation harbored Clostridia while none were recovered distally. In two other cases Clostridia were demonstrated only distal to the amputation site. Six cases yielded Clostridia both distal to and at the amputation site.

Table XV. Distribution of Clostridia and Aerobic Flora Recovered from Amputated Extremities

Case No.	Specimen	Tissue	Clostridia in Each Specimen	Aerobic Flora of All Specimens*
1	a	Leg: amputation site	<u>Cl. perfringens</u> ; <u>Cl. tetani</u> <u>Cl. cochlearium</u>	<u>beta strep.</u> <u>Strep. H-</u> <u>Staph H-</u>
	b	Leg: distal to amputation	Negative	<u>Staph H-</u> ; <u>Proteus sp.</u>
	c	Foot: muscle from plantar area	Negative	<u>Bacillus</u> , 4 spp
2	a	Leg: amputation site	Negative	<u>beta strep.</u>
	b	Leg: muscle distal to amputation	<u>Cl. lentoputrescens</u> <u>Cl. cochlearium</u>	<u>Strep. H-</u> <u>Corynebacterium sp.</u> ; <u>Bacillus sp.</u> <u>Proteus sp.</u>
3	a	Leg: amputation site	<u>Cl. sporogenes</u> ; <u>Cl. bif-</u> <u>fermentans</u> ;	<u>beta strep.</u> <u>Strep. H-</u> ; <u>Cor-</u>
	b	Leg: deep muscle near amputation site	<u>Cl. novyi</u> ; <u>Cl. sporogenes</u>	<u>ynebacterium sp.</u> <u>Lactobacillus sp.</u>
	c	Leg: deep muscle, between amp. site & foot	<u>Cl. bif fermentans</u> ; <u>Cl. spor-</u> <u>ogenes</u>	<u>Proteus sp.</u> <u>Bacillus sp.</u>
	d	Foot (muscle)	<u>Cl. sporogenes</u> ; <u>Cl. bifermen-</u> <u>tane</u>	<u>Sarcina lutea</u>
4	a	Leg: amputation site	<u>Cl. sporogenes</u>	<u>Bacillus sp.</u> <u>beta. strep.</u>
	b	Leg: posterior muscle, amputation site	<u>Cl. sporogenes</u>	<u>Corynebacter-</u> <u>ium sp.</u>
	c	Leg: deep muscle near amputation site	<u>Cl. sporogenes</u>	<u>Proteus sp.</u>
	d	Leg: exposed muscle near amputation site	Negative	
5	a	Leg: amputation site	<u>Cl. perfringens</u> ; <u>Cl. mul-</u> <u>tifermentans</u> ; <u>Cl. novyi</u> <u>Cl. sporogenes</u>	<u>beta strep.</u> <u>alpha strep.</u> <u>Strep. H-</u>
	b	Leg: muscle distal to amputation site	Negative	<u>Pseudomonas aer-</u> <u>uginosa</u> <u>Bacillus sp.</u>

Table XV. Distribution of Clostridia and Aerobic Flora Recovered from Amputated Extremities Continued

Case No.	Specimen	Tissue	Clostridia in Each Specimen	Aerobic Flora of All Specimens*
6	a	Leg: amputation site	Negative	beta strep.; alpha
	b	Leg: soleus muscle, distal amp. site		strep; Strep. H-; Staph. H-; Sarcina
	c	Leg: deep muscle near	<u>Cl. novyi</u> ; <u>Cl. fesceri</u>	<u>lutea</u> ; <u>Bacillus</u> sp.
7	a	Leg: near amputation site	<u>Cl. sporogenes</u>	<u>Bacillus</u> sp.
	b	Leg: middle portion (muscle)	<u>Cl. sporogenes</u> <u>Cl. perfringens</u>	<u>Corynebacterium</u> sp. Strep. H-
	c	Leg: distal portion (muscle)	<u>Cl. perfringens</u>	
8	a	Leg: amputation site	<u>Cl. novyi</u> ; <u>Cl. sporogenes</u> ; <u>Cl. multifementans</u>	beta strep. Strep. H.; <u>Bacillus</u> 5 spp.; <u>Sarcina</u> <u>lutea</u> ; <u>Pr. morgani</u> ; Yeast
	b	Foot: deep muscle	<u>Cl. sporogenes</u>	
9	a	Leg: soleus muscle near amp. site	<u>Cl. bifermentans</u>	beta strep.; Strep. H-; <u>Proteus morganii</u> ;
	b	Leg: gastrocnemius near amp. site	Negative	<u>Corynebacterium</u> sp. <u>Sarcina lutea</u> ; <u>Bacillus</u>
	c	Leg: muscle near amp. site but distal to a & b	<u>Cl. sporogenes</u>	<u>lus</u> sp.
	d	Leg: muscle distal to a and b, and posterior to c	<u>Cl. sporogenes</u> <u>Cl. novyi</u> ; <u>Cl. tertium</u>	
10	a	Leg: amputation site disarticulation knee	<u>Cl. perfringens</u> <u>Cl. multifementans</u> <u>Cl. sporogenes</u>	beta strep. Strep. H- <u>Bacillus</u> sp.
	b	Leg: amputation site knee joint	<u>Cl. multifementans</u>	
	c	Leg: muscle distal to amputation	<u>Cl. perfringens</u> <u>Cl. multifementans</u> <u>Cl. sporogenes</u> <u>Cl. bifermentans</u>	

* H/ = hemolytic
H- = non-hemolytic

Table XVI shows the frequency of isolation of one or more strains of toxigenic and non-toxigenic Clostridia in the same amputated extremity. In 15 cases (45%) only non-toxigenic species were present, while in 2 cases only toxigenic Clostridia were present. In the remaining 16 cases, toxigenic and non-toxigenic strains were both present, with non-toxigenic strains predominating.

Table XVI. Combinations of Clostridia Occurring in Amputation Specimens

	Number of Cases									
	1	0	1	2	0	1	2	2	1	
Toxigenic Species	1	0	1	2	0	1	2	2	1	
Non-toxigenic Species	0	1	1	0	2	2	1	2	2	
Total Observed	1	8	4	1	7	5	1	4	2	

Aerobic Flora of Wounds - War wound infections are always mixed and aerobic bacteria play a significant role in the biology of wounds. From 192 tissue specimens studied, 839 strains of aerobes were identified (Table XVII). For convenience, 13 strains of anaerobic streptococci are included. Most of these ultimately became micro-aerophilic. Ten genera of aerobes were distinguished. An average of 4.3 aerobic strains per specimen were recovered.

Table XVII. Aerobic Flora of Cultures from Wound and Tissue, 1952

		Total Aerobic Strains	% of Total Aerobes
Staphylococcus:			
H/S/M/C/*	5		
H/S/M/C-	23		
H/S/M-C-	10		
H/S-M-C-	1		
Hemolytic, unclassified	46		
Non-hemolytic	101		
		186	22.2
Streptococcus			
beta-hemolytic	92		
alpha-hemolytic	26		
non-hemolytic	160		
anaerobic, beta-hemolytic	6		
anaerobic, alpha-hemolytic	3		
anaerobic, non-hemolytic	4		
		291	34.6
Bacillus spp. (8 species)		182	21
Pseudomonas spp.		31	3.8
Proteus spp. (3 species)		25	2.9
Corynebacterium spp. (5 species)		62	7.4
Coliform:		62	7.4
Aerobacter aerogenoides	14		
Escherichia coli	11		
Paracolon spp.	32		
Alkaligenes sp.	5		

*H/ : Hemolytic S/ : Salt-resistant M/ : Mannite fermenting
C/ : Coagulase positive

H- : Non-hemolytic S- : Salt-sensitive M- : Mannite negative
C- : Coagulase negative

Of the potentially pathogenic aerobes the staphylococci and streptococci were most significant. Differences between amputation and soft tissue wound specimens included an increased ratio of coliform bacilli in the latter and an increased incidence of Pseudomonas and Proteus in the former. The incidence of anaerobic streptococci was undoubtedly higher than noted here; techniques of isolating these strains were subordinated to the major problem of recovering Clostridia.

STUDY OF ANAEROBES IN FRESHLY INCURRED WAR WOUNDS: Virtually all literature on war wounds has been based on cultures taken one or more days after wounding, (22), (23), (24). This laboratory has accumulated data on infected wounds and on extremities amputated because of gangrene (7). Evacuation procedures and combat circumstances seldom permit a systematic bacteriologic study of wounds within 6 hours of injury. During 1952, the static war situation with consequent shortening of the evacuation time to the Mobile Army Surgical Hospital level offered a unique opportunity for such observations. During August and September, cultures were taken during debridement at an average of $4\frac{1}{2}$ hours from time of wounding. Only 13 percent of the patients studied arrived more than 6 hours post-wounding. All patients received 300,000 or 600,000 units of procaine penicillin i.m. either on the field or at the battalion aid station.

Thirty-nine patients were studied. Tissue removed at debridement was cultured from 27; 6 had blood culture and culture of tissue. Another 6 had only blood culture. Tissue specimens were planted promptly with aseptic precaution in cooked heart medium in screw-capped tubes. Foreign bodies, including shell fragments and clothing in tissue, were cultured in a similar manner. All cultures were examined in Tokyo. Blood cultures were drawn immediately prior to operation, after cleansing the skin with merthiolate and alcohol.

Twenty-nine soil samples were collected near the MLR from dry rice paddies, hillsides, bunkers, communication trenches, and company areas. Cultures were initiated in cooked meat medium in the Tokyo laboratory, within 6 weeks after collection.

Technique for isolation has been described (6, 7). Enrichment (cooked meat) broth cultures were transferred to blood agar plates at 24 and 48 hours and at longer intervals. Spreading-inhibitors included chloral hydrate, sodium azide, sorbic acid and alcohol. Subcultures, aerobic control plates, and differentiating procedures including motility, indol, nitrate, milk, carbohydrates, and gelatin liquefaction, animal pathogenicity and protection tests with specific antiserum were performed. The acrolein reaction for presumptive recognition of Cl. perfringens, and new methods for inhibiting contaminating spreading growth were employed extensively and are discussed under a separate heading.

Nature of Injuries - The study consisted of 21 leg wounds ranging from penetrating wounds of soft tissue to compound fractures and traumatic amputations, 11 arm and shoulder wounds, and 8 trunk or head and neck injuries. Causes of wounds were as follows:

Artillery	6
Small arms fire	5
Shell fragments:	
Mortar and grenade	15
Mines and Trip flares ..	6
Unspecified	7

Table XVIII summarizes the number of strains and species recovered from these patients in the cases where cause of injury was known.

Specimens from shell-fragment wounds showed the highest rate (88%) of positive Clostridia cultures. Mortar, grenade, and land mine wounds were each positive in 65% of the specimens. Small arms wounds yielded Clostridia in 55% of specimens. Only 3 patients in this group failed to yield Clostridia. The average number of species per patient varied from 1.3 (small arms wounds) to 6.0 (land mines). The average number of species per specimen was similar for small arms, artillery, and land mines, but was somewhat higher with mortar and grenade injuries.

Table XVIII. Causes of Wounds and Clostridia Present in 32 Cases

Cause of Wound	No. of Patients	Patients / for Clostridia	Ave. No. Species per Patient	Specimens Total	Specimens / for Clostridia	No. of Strains Recovered	No. of Species	Ave. Species per Specimen
Small-arms	5	5	1.8	11	6	10	5	1.7
Artillery	6	5	3.0	18	16	29	8	1.7
Mortar and Grenade	15	14	4.8	47	31	67	17	2.16
Land mine	6	5	6.0	26	17	30	10	1.8
Total	32	29		102	70	136		

Fifteen blood cultures were taken from 12 of these severely wounded men. These blood cultures were positive for Clostridia in 11 patients and yielded 19 strains, including 4 species. There were also 4 aerobic strains of organism recovered. The aerobes were always found in mixed cultures with the anaerobes.

The anaerobic flora from samples of tissue, blood, and soil is summarized in Table XIX.

Table XIX. Anaerobic Flora of Wounds, Blood, and Soil Samples
August - September, 1952

Clostridium Species	Tissue*		SOURCES Blood		Soil		Total	
	No.	% of Total Strains	No.	% of Total Strains	No.	% of Total Strains	No.	% of Total Strains
<u>sporogenes</u>	40	29	11	58	16	20.8	67	28.8
<u>perfringens</u>	26	18.8			25	31.4	51	21.9
<u>bifermentans</u>	15	10.9	4	21	10	13.0	29	12.0
<u>sordelli</u>	4	2.8			1	1.3	5	2.1
<u>multifermentans</u>	11	8.0	1	5	12	15.6	24	10.3
<u>novyi</u>	10	7.2	3	16	3	3.9	16	6.8
<u>paraputrificum</u>	5	3.6			1	1.3	6	2.5
<u>tetanomorphum</u>	4	2.9			1	1.3	5	2.1
<u>butyricum</u>	3	2.2			4	5.2	7	3.0
<u>tertium</u>	3	2.2			1	1.3	4	1.7
<u>lentoputrescens</u>	3	2.2			1	1.3	4	1.7
<u>tetani</u>	3	2.2					3	1.2
<u>aerofaecitidum</u>	2	1.4					2	0.8
<u>histolyticum</u>	1	0.7					1	0.4
<u>carnis</u>	1	0.7			1	1.3	2	0.8
<u>cochlearium</u>	1	0.7			1	1.3	2	0.8
<u>putrificiens</u>	1	0.7					1	0.4
<u>sphenoides</u>	1	0.7					1	0.4
<u>atypical perfringens</u>	3**	2.2					3	1.2
Total	138		19		77		233	

* 5 specimens included embedded clothing in wounds; 1 specimen was a shell fragment

** Non-toxicogenic, non-sucrose fermenting or otherwise not conforming to typical Cl. perfringens pattern.

Ninety-four tissue specimens from 33 patients were cultured. Clostridia were recovered in 63 samples (67%). These positive cultures were from 30 of the patients, there being an average of 4 species per patient and 2 strains per specimen. All cloth samples removed from wounds yielded Clostridia, although the tissue from one of these wounds did not. Predominant among the 19 species recovered were Cl. sporogenes, perfringens, bifermentans, multifermentans, and novyi; 66% of wound strains and 80.8% of soil strains were among these species. Toxigenic species included perfringens, novyi, and sordelli. The comparison of tissue specimens with soil samples was interesting in that perfringens predominated in soil samples while sporogenes occurred more frequently in tissue. The absence of Cl. tetani from soil samples was unexpected. Cl. novyi was more common in tissues than in soil. The wide diversity of species from tissue was striking.

The presence of Clostridia in blood cultures of these recently wounded men was entirely unexpected. The immediate suggestion was that contamination was involved. However, it is improbable that such exotic flora should be recovered as contaminants. The predominance of Cl. sporogenes in blood cultures as compared to its incidence in soil, the absence of Cl. perfringens and the presence of Cl. novyi make it more probable that an early transient Clostridial bacteremia occurs in some seriously wounded men. Clostridial bacteremia has been observed in septic abortion (27) and it seems plausible that rapidly proliferating anaerobes in recent wounds might enter the blood stream.

It is generally assumed that soil, being heavily seeded with Clostridia, is the usual source of wound contamination. All 29 soil samples cultured were positive, yielding a total of 13 species and 77 strains of Clostridia. Twenty-five soil samples yielded Cl. perfringens. An average of 2.76 strains per soil sample were recovered, as compared with 2.0 strains per tissue sample. Since soil samples were cultured in the laboratory directly, instead of being shipped in cooked meat broth from Korea, a higher recovery rate of Clostridia would be expected.

Aerobic Flora of Wounds - A limited study of the aerobic flora of 90 tissue samples is summarized in Table XX. The cultures were made from the cooked meat broth used for anaerobic enrichment, and may hence reflect some degree of selection.

Table XX. Aerobic Flora of Freshly Incurred Wounds in Korea

	<u>Strains</u>	<u>% of Total Strains</u>
<u>alpha hemolytic streptococci</u>	10	5.0
<u>beta hemolytic streptococci</u>	1	.5
<u>Streptococci H-</u>	35	17.5
<u>Staphylococci H/M</u>	3	1.5
<u>Staphylococci H/M-</u>	15	7.5
<u>Staphylococci H-</u>	11	5.5
<u>Pseudomonas aeruginosa</u>	8	4.0
<u>Proteus sp.</u>	27	13.5
<u>Escherichia spp.</u>	19	9.5
<u>Aerobacter sp.</u>	27	13.5
<u>Paracolon sp.</u>	10	5.0
<u>Alcaligenes sp.</u>	3	1.5
<u>Bacillus spp.</u>	29	14.5
<u>Corynebacterium spp.</u>	2	1.0
Total	198	100.0
H/ : hemolytic M/ : mannite-fermenting		
H- : non-hemolytic M- : non-mannite fermenting		

Predominant aerobes included streptococci, staphylococci, Proteus, coliforms, and Bacillus species. The virtual absence of beta hemolytic streptococci and hemolytic mannite-fermenting staphylococci was conspicuous. Enterobacteriaceae comprised almost one-third of the flora. The predominance of gram-negative bacilli and paucity of hemolytic streptococci was in marked contrast to the flora of older wounds.

Topical Distribution of Clostridia in Tissues of Recent Wounds - The relationship between time of wounding, debridement, and the distribution of Clostridia in the tissue is complex. Summaries of totals of organisms present do not adequately portray the situation. Table XXI gives the findings in 18 seriously wounded men. The time noted represents the interval between wounding and debridement, and between first dose of penicillin and debridement.

Table XXI. Distribution of Anaerobic Flora in Tissues of Recent Wounds

Case No.	Injury, Cause and Location	Time in Hours		Specimen	Clostridia Recovered
		Wound to Culture	Pen. to Culture		
1	GSW: left shoulder	5½	2	a. Clothing, uniform	<u>Cl. sporogenes</u> <u>Cl. perfringens</u> <u>Cl. bifermentans</u>
				b. Tissue: wound of entrance	None
				c. Tissue: wound of exit	<u>Cl. sporogenes</u>
3	Mortar: leg	3½	3½	a. Blood	No growth
				b. Tissue, wound of entrance	<u>Cl. paraputrificum</u> <u>Cl. bifermentans</u>
				c. Deep muscle	<u>Cl. perfringens</u>
4	Mine: right leg, amputation; avulsed wound left thigh	2½	1½	a. Blood	No growth
				b. Clothing in wound	<u>Cl. perfringens</u> <u>Cl. multif fermentans</u>
				c. Muscle, amp. site	<u>Cl. bifermentans</u>
				d. Deep tissue, amp. site	No growth
				e. L. thigh, deep	<u>Cl. sporogenes</u>
5	SFW: head and extremities	2½	1½	a. L. thigh, deep muscle	<u>Cl. sporogenes</u>
				b. R. calf, elective amputation	Negative
6	SFW: head; leg (died at 8 hrs)	1½	1	Blood	<u>Cl. bifermentans</u> <u>Cl. perfringens</u> <u>Cl. multif fermentans</u> <u>Cl. bifermentans</u> <u>Cl. sordelli</u>
				a. L. thigh, deep muscle	<u>Cl. multif fermentans</u> <u>Cl. bifermentans</u> <u>Cl. sordelli</u>
				b. L. thigh, sub-cut. tissue	<u>Cl. multif fermentans</u> <u>Cl. bifermentans</u>

Table XXI. Distribution of Anaerobic Flora in Tissues of Recent Wounds
Continued

Case No.	Injury, Cause and Location	Time in Hours Wound to Culture	Pen. to Culture	Specimen	Clostridia Recovered
7	Grenade: traumatic amputation of leg	4	2½	a. Deep muscle, amp. site b. Deep muscle, traumatic amp. site c. Muscle from traumatic amp. site	No growth <u>Cl. perfringens</u> <u>Cl. sporogenes</u> <u>Cl. bifermentans</u> <u>Cl. sordelli</u>
9	SFW: penetrating both thighs	8	1	a. Deep muscle, l. thigh b. Blood c. Deep muscle, l. thigh d. Deep muscle, r. thigh e. Deep muscle, r. thigh	<u>Cl. perfringens</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u> <u>Cl. perfringens</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u> <u>Cl. sporogenes</u> <u>Cl. sporogenes</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u>
		4 days post-wounding		a. Deep muscle, l. thigh b. Deep muscle, l. thigh	<u>Cl. bifermentans</u> <u>Cl. capitovalis</u> <u>Cl. sporogenes</u>
		9 days post-wounding		a. Deep muscle, l. thigh b. Deep muscle, l. thigh	<u>Cl. perfringens</u> <u>Cl. perfringens</u>
10	Trip flare; traumatic amp. hand	3½	3	a. Deep muscle, wrist b. Subcut. tissue	No growth <u>Cl. tetanomorphum</u>
11	SFW: l thigh	33/4	2	a. Subcut. tissue b. Deep muscle c. Clothing embedded in tissue	<u>Cl. perfringens</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u> <u>Cl. sporogenes</u>
13	Trip flare, l.	4½	0	a. Subcut. tissue b. Deep muscle	<u>Cl. novyi</u> <u>Cl. sporogenes</u>
17	Mortar: l. leg	3	1½	a. Deep muscle, calf b. Deep muscle, l. thigh	<u>Cl. tetanomorphum</u> <u>Cl. cochlearium</u> <u>Cl. putrificans</u> <u>Cl. sporogenes</u> <u>Cl. lentoputrescens</u> <u>Cl. tetani</u> <u>Cl. multif fermentans</u> <u>Cl. tetanomorphum</u>
18	Mine: traumatic amputation leg; avulsed wound left leg	3½	23/4	a. Subcut. tissue, r. leg b. Deep muscle, r. leg c. Subcut. tissue, r. leg d. Deep muscle, r. leg	<u>Cl. perfringens</u> <u>Cl. multif fermentans</u> <u>Cl. sporogenes</u> <u>Cl. sporogenes</u> <u>Cl. perfringens</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u>

Table XXI. Distribution of Anaerobic Flora in Tissues of Recent Wounds
Continued

Case No.	Injury, Cause and Location	Time in Hours		Specimen	Clostridia Recovered
		Wound to Culture	Pen. to Culture		
18 Continued					
				e. Subcut. tissue, 1 leg	<u>Cl. sporogenes</u>
				f. Deep muscle, 1. leg	No growth
				g. Subcut. tissue, traumatic amputation site	<u>Cl. bifermentans</u> <u>Cl. tetanomorphum</u>
				h. Deep muscle, 1. leg	<u>Cl. perfringens</u> <u>Cl. tetani</u> <u>Cl. multifementans</u>
				i. Deep muscle, 1. leg	No growth
				j. Deep muscle, r. leg	No growth
20	Carbine, 1. arm	5½	2	a. Subcut. tissue, 1 arm	<u>Cl. perfringens</u> <u>Cl. multifementans</u> <u>Cl. butyricum</u>
23	Mortar: Chest	5½	4	a. Subcut. tissue, r. chest	<u>Cl. aerofœtidum</u>
				b. Deep muscle, r. chest	<u>Cl. perfringens</u> <u>Cl. paraptrophicum</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u>
				c. Subcut. tissue, r. forearm	<u>Cl. tertium</u> <u>Cl. multifementans</u> <u>Cl. sphenoides</u>
				d. Deep muscle, r. forearm	<u>Cl. novyi</u> <u>Cl. sporogenes</u>
25	Mortar: buttock; leg amputation	8½	5	a. Subcut. tissue, r. thigh	<u>Cl. sporogenes</u>
				b. Deep muscle, r. thigh	<u>Cl. histolyticum</u> <u>Cl. novyi</u> <u>Cl. paraptrophicum</u>
				c. Deep muscle, lower r leg	<u>Cl. perfringens</u>
				d. Deep muscle, lower r leg	<u>Cl. perfringens</u>
				e. Deep muscle, amp. site	<u>Cl. perfringens</u>
				f. Deep muscle, amp. site	<u>Cl. perfringens</u> <u>Cl. bifermentans</u> <u>Cl. sporogenes</u>
26	Small arms and SFW upper 1. arm and face	4 6	1 3	a. Blood	<u>Cl. novyi</u> <u>Cl. sporogenes</u>
				b. Deep muscle, 1. arm	No growth

Table XXI. Distribution of Anaerobic Flora in Tissues of Recent Wounds Continued

Case No.	Injury, Cause and Location	Time in Hours		Specimen	Clostridia Recovered
		Wound to Culture	Pen. to Culture		
27	GSW, l. hand SIW	4½	3	a. Subcut. tissue wound of entrance	No growth
				b. Subcut. tissue wound	<u>Cl. multiferm-tans</u> <u>Cl. butyricum</u>
30	Mortar: legs	4	1	a,b,c: Specimens from leg, ankle, thigh	No growth
				d, e: Specimens from opposite leg	No growth

Injuries included 10 shell fragment wounds or mortar injuries, 5 mines or grenades, and three gunshot wounds. The juxtaposition of debrided tissue samples devoid of anaerobes and samples containing several species may be noted in cases 4, 7, 18, and 27. In most instances, however, Clostridia were widely distributed in tissues adjacent to the wound. Cl. sporogenes and perfringens frequently occurred together, and Cl. bifermentans and sordelli were demonstrated in a single block of tissue in several instances. The distinction between the latter 2 species was made despite frequent allusions in the literature to Cl. sordelli as a toxigenic variety of Cl. bifermentans. Biologically, these species are no more closely related than are Cl. sporogenes and Cl. novyi, and their differentiation is believed justified.

Blood cultures from four cases included in the table showed Clostridia in 3 instances, with one, two, and three species present in the respective cases. One case, #6, was a head injury in which death occurred 6 hours post-operatively. In this case, Cl. bifermentans was recovered in the wound and in the blood culture. Case #9 had 3 species present in blood and in several wounds. Case 25 suffering head and arm wounds, yielded two species in blood culture; the single tissue specimen did not harbor demonstrable Clostridia.

Sensitivity of Recently Isolated Clostridia to Antibiotics - It has been assumed that antibiotic therapy plays a vital role in preventing major Clostridial involvement in contaminated wounds, but little information on sensitivity of Clostridial strains from wounds has been recorded. Approximately 480 anaerobes from the current study were tested for such sensitivity. Attempts to use a plate diffusion technique were unsuccessful due to frequent growth failures. A tube method was employed using a 1:100 dilution of an 18-hour thioglycollate broth culture in varying dilutions of antibiotic. The growth end point was read at 20 hours. Sensitivity tests were made within one week of isolation, usually prior to final identification. Minimum inhibiting concentrations of penicillin, aureomycin, terramycin, and chloromycetin for 14 of the 19 species recovered in this study are shown in Tables XXII, XXIII, XXIV, and XXV. Sensitivity of most strains to penicillin, aureomycin, and terramycin fell within a narrow range. Reaction to Chloromycetin varied more widely. In several cases, multiple strains from a given patient were tested prior to identification of the species, which accounts for the proportionately large number of perfringens and sporogenes strains tested. The majority of strains of 14 species tested were inhibited by penicillin in ranges from 0.1 unit/ml to 1.0 unit/ml. None were inhibited by less than 0.05 units/ml. and only 29 of 453 strains withstood 2 units or more of penicillin. Of the potentially important organisms, penicillin-resistant strains of perfringens, novyi, bifermentans, and sporogenes, were observed. With aureomycin the range of sensitivity was somewhat greater than with penicillin, but most strains were inhibited by at least 0.5 mcg/ml. As with penicillin, a potentially resistant group of strains was present. A similar picture with a still wider range of sensitivity (from 0.5 mcg/ml) was found with terramycin. Ten percent

Table XXII. Sensitivity of Recently Isolated Clostridia to Penicillin

Species	Minimum Inhibiting Concentration (unit/ml)									Resists Maximum	Total Strains
	0.25	.05	.1	.25	.5	1.0	2.0	3.0	5.0		
<u>Cl. perfringens</u>	-	8	28	56	55	25	2	4	-	6	184
<u>Cl. sporogenes</u>	-	4	9	21	58	24	9	1	-	6	132
<u>Cl. bifermentans</u>	-	7	8	11	21	7	1	-	-	2	57
<u>Cl. multifementans</u>	-	4	9	6	7	5	2	2	1	-	36
<u>Cl. paraputrificum</u>	-	-	5	1	3	-	2	-	-	3	14
<u>Cl. tetanomorphum</u>	-	-	1	3	1	1	1	-	1	-	8
<u>Cl. novyi</u>	-	1	-	2	4	1	-	-	-	1	9
<u>Cl. butyricum</u>	-	-	2	2	-	-	-	-	-	-	4
<u>Cl. lentoputrescens</u>	-	-	-	-	-	-	-	-	-	1	2
<u>Cl. tertium</u>	-	-	-	-	-	1	1	-	-	-	2
<u>Cl. tetani</u>	-	-	1	1	-	-	-	-	-	-	2
<u>Cl. sordelli</u>	-	-	-	-	1	-	-	-	-	-	1
<u>Cl. carnis</u>	-	-	-	-	-	1	-	-	-	-	1
<u>Cl. aerofotidum</u>	-	-	-	-	-	-	-	-	-	1	1
Total Strains	-	24	64	104	150	65	18	7	2	20	453

Table XXIII. Sensitivity of Clostridia to Aureomycin

Species	Minimum Inhibiting Concentrations (mcg/ml)							Resists Maximum	Total Strains
	.01	0.25	.05	.1	.25	.5	1.0		
<u>Cl. perfringens</u>	-	1	16	56	80	23	9	4	189
<u>Cl. sporogenes</u>	-	1	7	46	51	11	12	4	132
<u>Cl. bifermentans</u>	-	1	7	12	24	9	7	5	65
<u>Cl. multifementans</u>	-	3	6	14	16	6	2	-	47
<u>Cl. paraputrificum</u>	-	-	1	2	1	1	1	6	12
<u>Cl. tetanomorphum</u>	-	-	-	-	4	3	1	3	11
<u>Cl. novyi</u>	-	-	-	3	3	1	1	1	9
<u>Cl. butyricum</u>	-	-	2	3	-	2	-	-	7
<u>Cl. lentoputrescens</u>	-	-	1	-	-	-	-	-	1
<u>Cl. tertium</u>	-	-	-	-	-	1	-	-	1
<u>Cl. tetani</u>	-	-	-	1	1	-	-	-	2
<u>Cl. cochlearium</u>	-	-	-	-	1	-	-	-	1
<u>Cl. sordelli</u>	-	-	1	-	-	-	-	-	1
<u>Cl. carnis</u>	-	-	1	-	-	-	-	-	1
<u>Cl. aerofotidum</u>	-	-	-	-	1	-	-	-	1
Total Strains	-	6	42	137	183	57	33	23	480

of the strains tested were resistant to 20 mcg/ml of chloromycetin although the majority were inhibited by 5 mcg/ml or less.

No significant variations in sensitivity were demonstrated between species. However, the range of sensitivity of different strains of multifementans, tetanomorphum, and paraputrificum was wider than that for other species. The range of antibiotic concentrations at which 80% of the 5 predominant species were inhibited were determined (Table XXVI).

When the composite sensitivity of all strains tested was assessed in terms of percent of total strains inhibited by a given concentration of antibiotic, the comparative results indicated differences in the type of effect exerted by the four antibiotics. Graph II (Figure 2) shows the results of tests with penicillin, aureomycin, terramycin, and

Table XXIV. Sensitivity of Clostridia to Terramycin

Species	Minimum Inhibiting Concentrations (mcg/ml)									Resists Maximum	Total Strains
	0.25	.05	.1	.25	.5	1.0	2.0	3.0	5.0		
<i>Cl. perfringens</i>	-	1	24	78	49	20	17	1	-	3	193
<i>Cl. sporogenes</i>	-	3	12	72	29	8	5	2	2	2	135
<i>Cl. bifermentans</i>	-	5	6	28	16	3	-	-	-	2	60
<i>Cl. multifementans</i>	-	4	5	17	14	2	2	1	-	-	45
<i>Cl. paraputrificum</i>	-	-	3	2	1	2	1	-	-	5	14
<i>Cl. tetanomorphum</i>	-	-	-	-	5	1	-	-	-	3	9
<i>Cl. novyi</i>	-	-	-	4	1	1	-	-	-	1	7
<i>Cl. butyricum</i>	-	-	-	3	3	-	-	-	-	-	6
<i>Cl. lentoputrescens</i>	-	-	-	1	-	-	-	-	-	-	1
<i>Cl. tertium</i>	-	-	-	2	-	-	-	-	-	-	2
<i>Cl. tetani</i>	-	-	1	-	1	-	-	-	-	-	2
<i>Cl. cochlearium</i>	-	-	-	-	2	-	-	-	-	-	2
<i>Cl. sordelli</i>	-	-	-	-	1	-	-	-	-	-	1
<i>Cl. carnis</i>	-	-	-	-	1	-	-	-	-	-	1
<i>Cl. aerofotidum</i>	-	-	-	-	1	-	-	-	-	-	1
Total Strains	-	13	51	209	124	37	35	4	2	16	479

Table XXV. Sensitivity of Clostridia to Chloromycetin

Species	Minimum Inhibiting Concentrations (mcg/ml)								Resists Maximum	Total Strains
	1.0	2.5	5.0	8.0	10.0	12.0	15.0	20.0		
<i>Cl. perfringens</i>	1	40	53	38	21	3	1	5	25	186
<i>Cl. sporogenes</i>	1	36	41	20	17	1	2	1	8	126
<i>Cl. bifermentans</i>	-	26	21	7	2	-	2	2	3	63
<i>Cl. multifementans</i>	-	17	12	7	1	-	1	-	5	43
<i>Cl. paraputrificum</i>	-	3	4	1	3	-	-	-	1	12
<i>Cl. tetanomorphum</i>	-	4	1	3	5	-	-	-	-	13
<i>Cl. novyi</i>	-	2	1	1	2	-	-	-	2	8
<i>Cl. butyricum</i>	-	3	1	-	-	-	-	-	-	4
<i>Cl. lentoputrescens</i>	-	1	-	-	-	-	-	-	1	2
<i>Cl. tertium</i>	-	1	1	-	-	-	-	-	-	2
<i>Cl. tetani</i>	-	-	2	2	-	-	-	-	-	4
<i>Cl. cochlearium</i>	-	-	1	-	-	-	-	-	-	1
<i>Cl. sordelli</i>	-	1	1	-	-	-	-	-	-	2
<i>Cl. aerofotidum</i>	-	-	1	-	-	-	-	-	-	1
Total Strains	2	135	148	79	51	4	6	8	45	467

chloromycetin. At the highest dilution of aureomycin tested, no indication of a distinct group of resistant forms appeared. However, the results for terramycin and penicillin show a levelling off above the concentration at which 90% of the strains were inhibited. Increases beyond 1 unit of penicillin or 1 mcg. of terramycin per ml. failed to inhibit a corresponding proportion of the strains, and a projection of the curves indicated that a significant number of extremely resistant strains were present in each case. Chloromycetin behaved in a more quantitative manner, and at the highest concentration employed the curve indicated a probably complete inhibitory level.

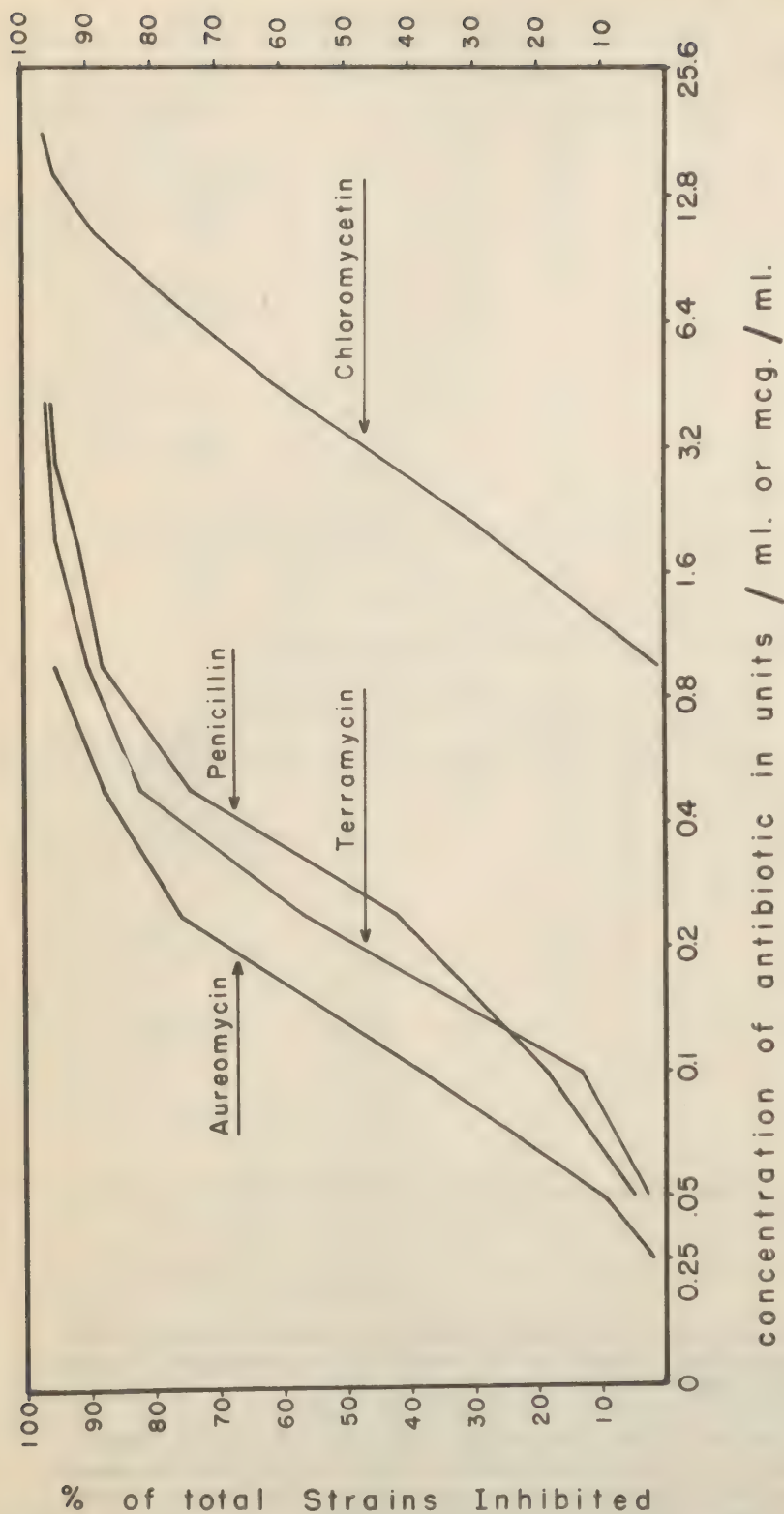


Figure 2. Sensitivity of Clostridia to Aureomycin, Terramycin, Penicillin, and Chloromycetin

Table XXVI. Range of Concentration of Antibiotics Which Inhibit More Than 80% of Clostridium perfringens, multifermentans, bi-fermentans, sporogenes, and novyi

Antibiotic	Species				
	Cl. perfringens	Cl. multifermentans	Cl. bifermentans	Cl. sporogenes	Cl. novyi
Terramycin					
Minimum Inhib. conc.	.1-1.0	.05-0.5	.05-0.5	.1-1.0	.25-1.0
No. of strains tested	193	45	60	135	7
% of total inhibited	88.7%	88.8%	91.7%	89.6%	85.7%
Aureomycin					
Minimum Inhib. conc.	.05-0.5	.05-0.5	.05-1.0	.1-1.0	.1-1.0
No. of strains tested	189	47	65	132	9
% pf total inhibited	92.8%	89.4%	90.8%	91.0%	88.8%
Penicillin					
Minimum Inhib. conc.	.1-1.0	.05-1.0	.05-1.0	.1-2.0	.05-1.0
No. of strains tested	184	36	57	132	9
% of total inhibited	89%	86.4%	94.7%	91.7%	88.8%
Chloromycetin					
Minimum Inhib. conc.		2.5-8.0	2.5-8.0	2.5-10.0	2.5-10.0
No. of strains Tested	186	43	63	126	8
% of total Inhibited	81.5%	83.7%	85.2%	90.0%	75.0%

Inhib. = inhibiting
Conc. = concentration

SORBIC ACID AN AID IN RAPID ISOLATION OF CLOSTRIDIA: Vaughn and Emard (28) reported growth of catalase-positive bacteria was inhibited by sorbic acid (2, 4 hexadienoic acid) at an acid pH. The possibility of using sorbic acid as an aid in isolation of Clostridia from wounds was investigated. Separation of anaerobes from Pseudomonas, Proteus, gram positive bacilli, and Staphylococci, is the basic problem. Chloral hydrate-sodium azide plates have not completely controlled the spreading of Proteus colonies in this laboratory (7). Colonies of hemolytic staphylococci are indistinguishable from smooth-phase Clostridium colonies on blood agar azide-chloral inhibition plates.

The glucose-yeast extract broth employed by Vaughn and Emard failed to support growth of pathogenic Clostridia. Likewise, the liver-infusion which they recommend was extremely difficult to prepare in an effective mixture with blood agar. Experiments were conducted with thioglycollate broth containing 0.175% agar, to which sorbic acid was added, to produce final contaminations ranging from 0.05% to 0.25%. The sorbic acid had no apparent effect on the medium except to lower the pH to a range of

5.6 to 5.8. This did not impede growth of Clostridia. Strains of gram-negative bacilli, and gram-positive cocci and bacilli were inhibited by a concentration of 0.15% of sorbic acid, as shown in Table XXVII.

Table XXVII. Effect of Concentration of Sorbic Acid Upon the Growth in Thioglycollate Broth of Aerobic Bacteria

Organism	No. of Strains	Sorbic Acid Concentration and Growth Results		
		0.5%	.10%	.15%
<u>Salmonella</u> sp.	10	+	+	-
<u>Shigella</u> sp.	10	+	-	-
Coliform strains	4	+	+	-
<u>Proteus</u> sp.	4	+	-	-
<u>Pseudomonas</u> sp.	5	+	+	+
<u>Bacillus</u> spp.	35	+	+	—*
Staphylococcus, Hemolytic	18	+	+	-
Staphylococcus, Non-hemolytic	15	+	+	-
Streptococcus, Non-hemolytic	10	+	+	-
Streptococcus, alpha hemolytic	5	+	+	+
Streptococcus, beta hemolytic	12	+	+	++

Growth readings are at 48 hours, incubation at 37°C.

* Several Bacillus strains were recoverable at 48 hrs., but did not grow in the presence of sorbic acid.

** Only Group D streptococcus strains survived.

+ Organisms growing and recovered upon subculture into nutrient medium

+ Organisms inhibited in growth by this concentration of sorbic acid, but remaining viable.

- Organisms non-viable when subcultured into nutrient medium

Among the strains tested only Pseudomonas and streptococci of Group D grew well in sorbic acid. In view of this encouraging result, 19 Clostridium species were further studied to make certain that no inhibition occurred in the presence of 0.15% sorbic acid. Growth under these conditions was sometimes slightly delayed, and the formation of gas from fermentable carbohydrates was suppressed. With these facts in mind, sorbic acid was incorporated in both thioglycollate broth and blood agar plates and applied to the isolation of Clostridia. Duplicates of enrichment broth cultures were used to maintain strains which might be inhibited by the acid, but thus far such inhibition has not been encountered.

This method has been more effective than all previous methods for inhibiting Proteus, Coliform bacilli, and Bacillus spp. The medium has been employed in over 500 isolation procedures without any indication that it has prevented recovery of Clostridium, and many strains recovered by its use would otherwise have been lost. It has greatly reduced the time required to recover pure cultures.

OBSERVATIONS ON CULTURAL REACTIONS OF CLOSTRIDIA AND USE OF POLYMXIN IN ISOLATION TECHNIQUE: The fastidious behavior of many strains of Clostridia on initial isolation, the interference of facultative anaerobes, spreading growth of aerobes, and mechanical difficulties in maintaining anaerobiosis for agar plates contribute to the uncertainty of results in Clostridia study. Since the outbreak of the Korean War, continued effort has been made to improve anaerobic culture techniques. Previous reports described the introduction of

semisolid thioglycollate broth for motility determination, nitrate reduction, and carbohydrate utilization, of sodium azide-chloral hydrate blood agar plates for isolation, and of the acrolein reaction for presumptive recognition of Cl. perfringens. Chopped heart medium (Robertson's broth) has been used as an enrichment broth, and for a holding medium for primary culture. During 1952, the acrolein reaction was evaluated and other critical factors in isolation, identification, and maintenance of cultures of anaerobes were assessed. Polymixin and Sorbic acid (q.v.) as an aid in isolation of strains were introduced.

Acrolein Reaction - The formation of acrolein (acrylic aldehyde) from glycerol by Cl. perfringens was regarded as a consistent reaction by Humphries (29) and by Heller (30). In this laboratory, a small percentage of acrolein-positive cultures consistently failed to yield Clostridium perfringens, while in some cases clostridia were recovered from acrolein-negative cultures.

The acrolein reaction is at present performed by inoculating 0.5 ml of an 18 hour thioglycollate broth culture into 2.5 ml of glycerol-thioglycollate broth, and incubating for 15 to 18 hours at 37°C. (In urgent cases, the test may be performed as soon as the indicator shows an acid reaction, or in as little as 6 hours). Prompt appearance, within 15 minutes, of the typical magenta to purple color on addition of Schiff's reagent, constitutes a positive reaction, while a delayed or weak color change is regarded as doubtful. Table XXVIII summarizes the results of tests on 455 anaerobic cultures.

Table XXVIII. Acrolein Reactions in 455 Anaerobic Cultures

Type of Specimen	Acrolein Positive	Acrolein Negative	<u>Cl. perfringens</u>		% of pos. reactors yielding <u>Cl. perfringens</u>	% of neg. reactors yielding <u>Cl. perfringens</u>
			Isolated	Not Isolated		
Tissue*	37		32	5	86.4%	
Tissue		175	8	167		4.5%
Wound study, Korea**	49		42	7	89.8%	
		84	3	81		3.5%
Food	4		4	0	100.0%	
		76	4	72		5.2%
Urine	0	13	1	12		7.6%
Miscellaneous	3	14	3	14	100.0%	
Totals	93		81	12	87.0%	
		362	16	346		4.6%

* A total of 10 doubtful reactions were recorded, of which 5 yielded Cl. perfringens and 5 did not.

** These cultures were all held at least 5 days prior to examination

Eighty-seven percent of cultures positive by the acrolein test yielded Cl. perfringens, which was recovered from only 5% of cultures giving a negative reaction. Cultures of Cl. tetani gave a weak acrolein reaction and one strain of Cl. tetanomorphum consistently gave a prompt positive reaction. The only previous recorded exception to the specificity of the test was by B. amaracrylus, an organism observed in wineries (31).

A possible explanation of aberrant acrolein reactions was noted in the behavior of 5 strains of Cl. perfringens which gave negative acrolein reaction on initial test. These organisms were pathogenic for guinea pigs, and when subsequently recovered from tissues of inoculated animals, gave positive acrolein reactions. It is possible that strains recovered from enrichment broths showing negative presumptive acrolein reactions, are low in virulence, and that this virulence is enhanced by animal passage.

Brewer Jar Lid Seals - In use of Brewer jars, the standard technique suggests a ring of plasticene as a seal for the jar lid (32). It has been repeatedly observed that adequate anaerobiasis is more apt to be maintained when a layer of "Cello-seal", or similar high pressure glass sealing compound is used. Minute leaks developing in the seal will almost always result in failure of growth of otherwise viable Clostridia. This technical problem has not been strongly emphasized in the literature, but probably accounts for many failures to isolate anaerobes. A series of several hundred recent isolates of Clostridia, during tests for antibiotic sensitivity, failed to grow on blood agar plates in Brewer jars while parallel cultures in other jars grew well. The only technical difference in these parallel tests was the plasticene seal on the unsatisfactory jars and the "Cello-seal" on the jars yielding growth.

Variations in Milk Fermentation - Presumptive recognition of Cl. perfringens by the "stormy fermentation" reaction is widely used (33). During 1952, strains of Cl. multifementans recovered from soil have shown a stormy fermentation closely resembling that of Cl. perfringens. Some tissue-strains, however, gave a delayed reaction, with acid, clotting and gas formation. When subcultured for 48 hours on blood agar and re-tested, a typical stormy fermentation occurred. The interference of Cl. multifementans in presumptive recognition of Cl. perfringens constitutes a potential source of error in wound bacteriology and is to be further studied.

Hydrogen Sulfide Formation and Toxigenicity of Cl. perfringens - Recently isolated salicin-negative Cl. perfringens strains frequently formed little or no H₂S. Such strains killed guinea pigs very rapidly. After several subcultures H₂S formation became more rapid and stronger and simultaneously the time required for the strains to kill guinea pigs lengthened to several days. At the same time gelatin liquefaction by the organism tended to become more rapid. This type of reaction has not been previously reported for Cl. perfringens.

Polymixin B in the Isolation of Clostridia - Pseudomonas is one of the principal genera of aerobic flora interfering with prompt isolation of Clostridia from wounds. Most inhibitory techniques, including sorbic acid (q.v.) are not effective against these organisms. Of 10 strains of Ps. aeruginosa nine were inhibited by Polymixin in a concentration of 5 mcg/ml. This antibiotic was then tested against 20 strains, including 13 species of recently isolated Clostridia. Cl. perfringens, multifementans, butyricum, aerofaetidum, carnis, novyi, sporogenes, bifementans, sordelli, histolyticum, lentoputrescens, tetani, and capitovialis readily tolerated concentrations of 10 mcg/ml. At 30 mcg/ml aerofaetidum, histolyticum, and novyi were slightly inhibited in growth. Polymixin B in blood agar is now being employed as an inhibitor with promising results.

TUBERCULOSIS - Cultures and Animal Inoculation - Comparison of techniques employed in laboratory examinations for tuberculosis is of value in the continued search for better methods of demonstrating the presence of tubercle bacilli. Table XXIX summarizes our results during 1952.

Table XXIX. Tuberculosis Examinations for 1952

Total clinical specimens examined	3,194
Cultures	9,041
Dubos' medium	3,355
Positive for AFB	320 (9.5%)
Corper's medium	3,453
Positive for AFB	171
Petragnini's medium	2,233
Positive for AFB	185 (8.2%)
Guinea pig inoculation from concentrates	1,742
TB positive	224 (12.8%)

Petragnini's medium was added to the routine culture procedure in March, 1952. It was 40% more effective in recovery of tubercle bacilli than Corper's, which has

been used in this laboratory since 1948 on the basis of comparative experiments at that time. The high recovery rate with Dubos' liquid medium would make it a superior substrate were it not for the large proportion of acid-fast bacilli which failed to produce lesions in guinea pigs, and in many instances did not grow when subcultured to solid medium. Some of these organisms were saprophytes; others undoubtedly represented nonviable tubercle bacilli. The recovery rate with guinea pig inoculation was higher than with any single culture medium; however, the total recovery rate with 3 media approximated that obtained with guinea pigs.

Neutral Red Test for Virulence - The cytochemical test for virulence of tubercle bacilli can be performed on growth from solid or liquid media (34), (35). With the former the advantage of the speed of the procedure is somewhat reduced by the time required to grow an adequate amount of culture, so that initial guinea pig inoculation usually gives pathogenicity results more quickly. With liquid cultures, a prompt, effective, in vitro assessment of virulence would offer a desirable improvement in speed over present animal inoculation methods.

Solid Media Cultures and N-R Test - One hundred and eighty-two freshly isolated strains of acid-fast bacilli were tested by the cytochemical procedure described by Dubos (35). Of these, 171 gave a positive test on first trial. Two strains were negative initially but positive on repetition. The initial negative results were probably due to insufficient organisms, since these two strains grew slowly. There appears to be a lower limit of cell volume below which the reaction with neutral red will not occur, even though acid-fast bacilli are observable microscopically.

Nine non-pathogenic strains grown on Petragnini's were tested. These included M. phlei, M. smegmatis, M. vara, and 3 strains which were proved to be non-pathogenic after recovery from sputum. Each strain gave a negative Neutral-red test.

Liquid Medium Cultures and Neutral-red Test - One hundred and eighteen positive initial acid-fast bacillus cultures in Dubos' medium were tested. Forty-four gave positive cytochemical tests while 74 were negative. Those cultures giving a positive reaction were all pathogenic and showed relatively large numbers of acid-fast bacilli, with noticeable "cording" growth (36). Of the 74 neutral-red negative strains, 50 were shown to be pathogenic for guinea pigs, while 15 were non-pathogenic. Those strains which gave negative neutral red reactions from liquid medium but which were pathogenic to guinea pigs gave positive neutral red reactions when recovered by subculture on solid media. Inability to determine pathogenicity and difficulty in obtaining subculture on solid media seriously limit the value of Dubos media on the neutral red test.

ENTERIC BACTERIOLOGY: The incidence of enteric infection among American troops and civilians in the Far East was low during 1952, despite an active epidemic in Japanese and extensive Shigellosis in Koreans. Studies were made of the distribution of types of Shigella and Salmonella, fluctuations in specific types in epidemics, unclassified strains, phase variation and group antigen structure of Shigella flexneri, and the agglutinin response in patients during a Shigellosis epidemic.

INCIDENCE AND DISTRIBUTION OF SHIGELLA TYPES IN JAPAN, OKINAWA AND KOREA, 1952 - Specific typing of Shigella was carried out following the procedures of Wheeler (37) Kauffman (38), Edwards and Ewing (39) and Madsen (52) on antigenic structure. Reviews of literature (7) have shown little recent information regarding species and type incidence in the Far East (41). Observations have included study of distribution patterns in indigenous populations and U.S. personnel. In 1950 (42) Sh. flexneri 2a was predominant in Tokyo, representing 40% of strains isolated, while Sh. sonnei comprised 35%. This incidence resembled that reported in Europe (43), India (44), and the U.S. (45) within the past 12 years.

During 1952, 6,485 specimens were examined, including 1,981 stool and rectal swab cultures. From these, 4,284 strains were identified as Shigella or Alkalescens-Dispar species. This recovery rate of 66%, exclusive of Salmonella strains, reflected reasonably effective screening on the part of installations submitting cultures.

Primary isolations from stools were made on SS and MacConkey's agar, while KIA slants were plated to MacConkey's to assure purity of culture. Lactose-sucrose enrichment broth was inoculated, incubated 4 to 6 hours, and negative reactors used to inoculate KIA slants, motility agar, citrate agar, urease medium and mannitol. When negative for fermentation at 24 hours, the broth was used for an indol test. Screening criteria for *Shigella* included glucose utilization, absence of prompt fermentation of sucrose, lactose, or salicin, and negative reactions for citrate utilization, H₂S, motility and urease formation.

Serologic typing by the slide method was routinely done, with questionable reactions confirmed by tube agglutination. Typing sera were prepared in this laboratory from strains obtained from the Army Medical Service Graduate School, the Communicable Disease Center, and other authoritative sources, and followed the basic pattern described by Wheeler (37) and Kauffman (38). Group factors were extensively used in identifications. Comparisons with sera supplied by Army Medical Service Graduate School were made frequently.

Four distinct population groups were observed during 1952: Americans in Japan, Japanese citizens in Tokyo, POW's and some US troops in Korea, and American personnel on Okinawa. Shigellosis was present on an epidemic scale in Korea. There were 87,000 cases reported among Japanese in Tokyo during the summer. Cultures on Japanese were taken weekly at a contagious disease hospital. Cases occurred sporadically among Americans in Japan and the Ryukyus. Table XXX gives the distribution of the predominant types of *Shigella* in these groups of patients, and Table XXXI the distribution of all recovered *Shigella*. The predominant cause of Shigellosis in Japan, Korea, and Okinawa was *Sh. flexneri*, which is consistent with the worldwide importance of this species in bacillary dysentery (45). Incidence of *Sh. dysenteriae* strains fell below the 1951 level, and this group was of only academic interest in Japan. *Sh. dysenteriae* 2 (*ambigua*) appeared sporadically. Recovery of *boydii* strains was less frequent than in previous years, and this species remained a curiosity in the Far East. An organism of importance not listed in the table is the paracolon with *Shigella dysenteriae* 3 (Q1167) factors. This organism was consistently recovered in a small percentage of dysentery cases in Tokyo, and in 5 cases of diarrhea in Americans. It appears to be definitely pathogenic. *Sh. sonnei* has in the past been of importance in Japan. It showed a marked decrease in incidence in 1951 but increased slightly in 1952. All strains of *Sh. sonnei* recovered showed both forms I and II directly upon isolation.

Table XXX. Predominant *Shigella* Types in 4 Major Population Groups in the Far East, 1952

	Sh. flex. 2a	Sh. flex. 2b	Sh. flex. 3	Sh. flex. 4	Sh. flex. 5	Sh. sonnei
Japanese (Tokyo)	29.2%	37.9%	8.0%	2.2%	0	10.5%
Americans (Tokyo)	28.4%	13.6%	7.4%	2.5%	1.2%	24.7%
Americans (Ryukyus)	22.2%	4.0%	15.1%	5.3%	0	16.4%
POW & Americans (Korea)	15.1%	1.18%	12.6%	28.2%	21.8%	1.48%

The summary shows the dissimilarity between distribution of *Shigella* in Japan and Korea despite the frequent communication between the two areas. *Sh. flexneri* 2b, predominant in Japanese, is of negligible incidence in Korea; 2a is far less frequent in Korea than it is in Japan, and *Sh. sonnei* is quite rare in Korea.

Americans in Japan showed a *Sh. flexneri* 2b incidence of approximately one-third that of the Japanese. *Sh. flexneri* 2b was rare in Okinawa. The type 2a incidence was

Table XXXI. *Shigella* Types in Japan, Korea, and Ryukyus, 1952

	Japan				Ryukyus		Korea		Total	
	Americans		Japanese							
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Sh. dysenteriae</i> 1	-	-	-	-	-	-	66	1.92	66	1.55
<i>Sh. dysenteriae</i> 2	2	2.5	6	1.0	4	2.6	177	5.15	189	4.44
<i>Sh. dysenteriae</i> 5	-	-	-	-	1	0.7	-	-	1	0.02
<i>Sh. dysenteriae</i> 6	-	-	-	-	-	-	1	0.02	1	0.02
<i>Sh. flexneri</i> 1a	1	1.2	1	0.2	14	9.2	244	7.11	260	6.10
<i>Sh. flexneri</i> 1b	1	1.2	51	8.5	32	20.5	79	2.30	163	3.82
<i>Sh. flexneri</i> 2a	23	28.4	174	29.2	34	22.2	517	15.10	748	17.53
<i>Sh. flexneri</i> 2b	11	13.6	226	37.9	6	4.0	41	1.18	284	6.65
<i>Sh. flexneri</i> 3	6	7.4	48	8.0	23	15.1	431	12.60	508	11.90
<i>Sh. flexneri</i> 4	2	2.5	14	2.2	8	5.3	966	28.20	990	23.20
<i>Sh. rabaulensis</i>	-	-	1	0.2	-	-	4	0.11	5	0.12
(<i>flexneri</i> 4c)*										
<i>Sh. flexneri</i> 5	1	1.2	-	-	-	-	750	21.80	751	17.60
<i>Sh. flexneri</i> 6	-	-	-	-	-	-	1	0.02	1	0.02
(<i>manchester</i>)										
<i>Sh. flexneri</i> 6	-	-	-	-	-	-	2	0.05	2	0.05
<i>Sh. flexneri</i> var "X"	-	-	6	1.0	-	-	26	0.75	32	0.75
<i>Sh. flexneri</i> var "Y"	-	-	6	1.0	3	2.0	45	1.31	54	1.28
<i>Sh. boydii</i> 1	-	-	-	-	-	-	8	0.23	8	0.19
<i>Sh. boydii</i> 5	-	-	-	-	-	-	7	0.20	7	0.17
<i>Sh. sonnei</i> I. II	20	24.7	63	10.5	25	16.4	51	1.48	159	3.74
A - D 1	5	6.2	-	-	-	-	2	0.05	7	0.17
A - D 2	5	6.2	2	0.3	2	1.3	6	0.17	15	0.35
A - D 3	3	3.7	-	-	1	0.7	8	0.23	12	0.28
A - D 4	1	1.2	-	-	-	-	1	0.02	2	0.05
Total	81	100	597	100	153	100	3433	100	4264	

* Identity of this "type" remains debatable

Note: 3 *Shigella flexneri* 4 were received from MAAG (Formosa)

comparable for Americans in Japan and Okinawa, and for Japanese. *Sh. flexneri* 3 was twice as common on Okinawa as in Americans in Tokyo. *Sh. sonnei* was over twice as frequent among Americans as among Japanese in Tokyo. The proportionately high incidence of *Sh. flexneri* "X" among Japanese may be linked with the incidence of *Sh. flexneri* 2b, since the most probable source of "X" is as a loss-variant of 2b (40). The similarity of *Shigella* flora of Americans in Japan and Okinawa and the differences between Americans and Japanese in the same community suggest that the Americans retain at least some of the flora typical of the United States, modified, but not entirely replaced by the common flora of Japan. *Sh. flexneri* 5 is rare in Japan, yet it was very common among the Koreans. Among strains not shown in Table XXX, *Sh. flexneri* 6 was almost non-existent in the Far East. This type is of considerable importance in America and Europe. The A-D group was predominantly confined to Americans.

The Korean cases furnished the only significant numbers of *Sh. dysenteriae* types. Type 1 occurred only there. The incidence of *Sh. flexneri* 1a in Korea resembled that seen on Okinawa. This type was almost non-existent in Japan. *Sh. flexneri* 2b was conspicuously rare in Korea, in contrast to its predominance among Japanese. Infections in Korea were still predominantly *Shigella flexneri* 4 in 1952, although the proportion of type 5 rose strikingly and type 3 assumed greater importance. The low incidence of other types was in contrast to Japan and Okinawa, especially the absence of *Sh. sonnei*, which is a conspicuous feature of American and European dysentery. The incidence of *Sh. flexneri* "Y" in Korea reflected the concentration of type 4a, of which these "Y"

strains may be loss-variants. All type "Y" strains were subcultured and 20 colonies of each were checked for specific phase factors, before they were designated as authentic non-specific strains.

Monthly Variations in Principal Shigella Type in Japan and Korea - The rise and fall of different types of causative organisms in bacillary dysentery epidemics is not well-documented, and the current study permitted a brief investigation of this phenomenon. The proportionate distribution of the predominant Shigella in the Japan epidemic is shown in Figure 3. The increase in type 2b and the complete disappearance of type 3 during the summer are of interest. The case rate during the year rose to its peak in August and remained high until late November.

In Korea (Table XXXII) the predominant type Sh. flexneri 4a, remained at about 30% to 35% for 4 months, fell to 6.9% in August, then resumed its prominence in October. (A fundamental change in identify of Sh. flexneri 4 types in Korea is pointed out in a later section). The incidence of Sh. flexneri 5 tended to follow that of 4a. Type 2a constituted about 10% of the total Shigella for the first 4 months, then increased in incidence. In August and September it was the predominant type, and continued to occur frequently through December. It became one of the dominant types in this epidemic. The occurrence of Sh. dysenteriae 2 was exotic; it appeared in not over 8% of cases, suddenly rose to 51% in June, then almost disappeared. A situation not shown in Table XXXII was the abrupt and almost total disappearance of Sh. flexneri 1b in Korea after April. Of 78 strains recovered, only 2 were found during the last 8 months in 1952. The Shigella dysenteriae types of the Sachs-Large group and of Sh. boydii were rare in the Far East during 1952.

Table XXXII. Monthly Variation of Shigella in Korea

Month	<u>Shigella dysenteriae</u>		<u>Shigella flexneri 2a</u>		<u>Shigella flexneri 3</u>		<u>Shigella flexneri 4</u>		<u>Shigella flexneri 5</u>		% of Total Strains
	No.	%	No.	%	No.	%	No.	%	No.	%	
January	39	6.5	49	8.2	90	15.1	202	33.9	87	14.5	79.2
February	22	5.6	29	7.4	64	16.4	117	30.0	104	26.6	86.0
March	26	3.1	90	10.5	130	15.4	286	24.0	217	25.8	88.8
April	26	8.0	47	14.5	33	10.2	101	31.3	60	18.6	82.6
May	1	2.7	9	24.4	4	10.8	9	24.4	8	21.6	83.9
June	28	51.0	1	1.8	4	7.3	6	10.9	11	20.0	91.0
July	22	18.0	16	13.7	3	2.6	17	14.5	5	4.2	53.0
August	3	3.0	46	45.5	10	9.9	7	6.9	15	14.8	80.1
September	0	0	36	26.3	24	17.5	27	19.4	27	19.4	82.6
October	0	0	23	18.4	12	9.6	53	42.5	23	18.4	88.9
November	8	1.5	137	26.7	48	9.4	92	18.0	150	29.3	84.9
December	2	1.3	34	22.5	9	6.0	39	25.8	43	28.5	84.1
Total	177	5.2	517	15.5	431	12.7	956	28.2	759	22.2	83.8

Shigella flexneri 4: Mannite Fermentation - Although Sh. flexneri 4 is widely regarded as predominantly mannite-fermenting (46), all 23 strains recovered from Japanese over a two year period were mannite-negative. Japanese investigators (48) have reported only mannite-negative strains in Japan. During 1951, all of the 1339 strains received from Korea were mannite-positive. In March, 1952, mannite-negative strains were observed from Korea for the first time. The subsequent monthly fluctuation in the ratio of mannite-positive to negative types is summarized in Table XXXIII.

An annual average of 15% of mannite-negative strains were found in this previously homogeneous group of dysentery bacilli. The incidence calculated from March (when they first appeared) was 25%. There was a marked rise in incidence during July and August.

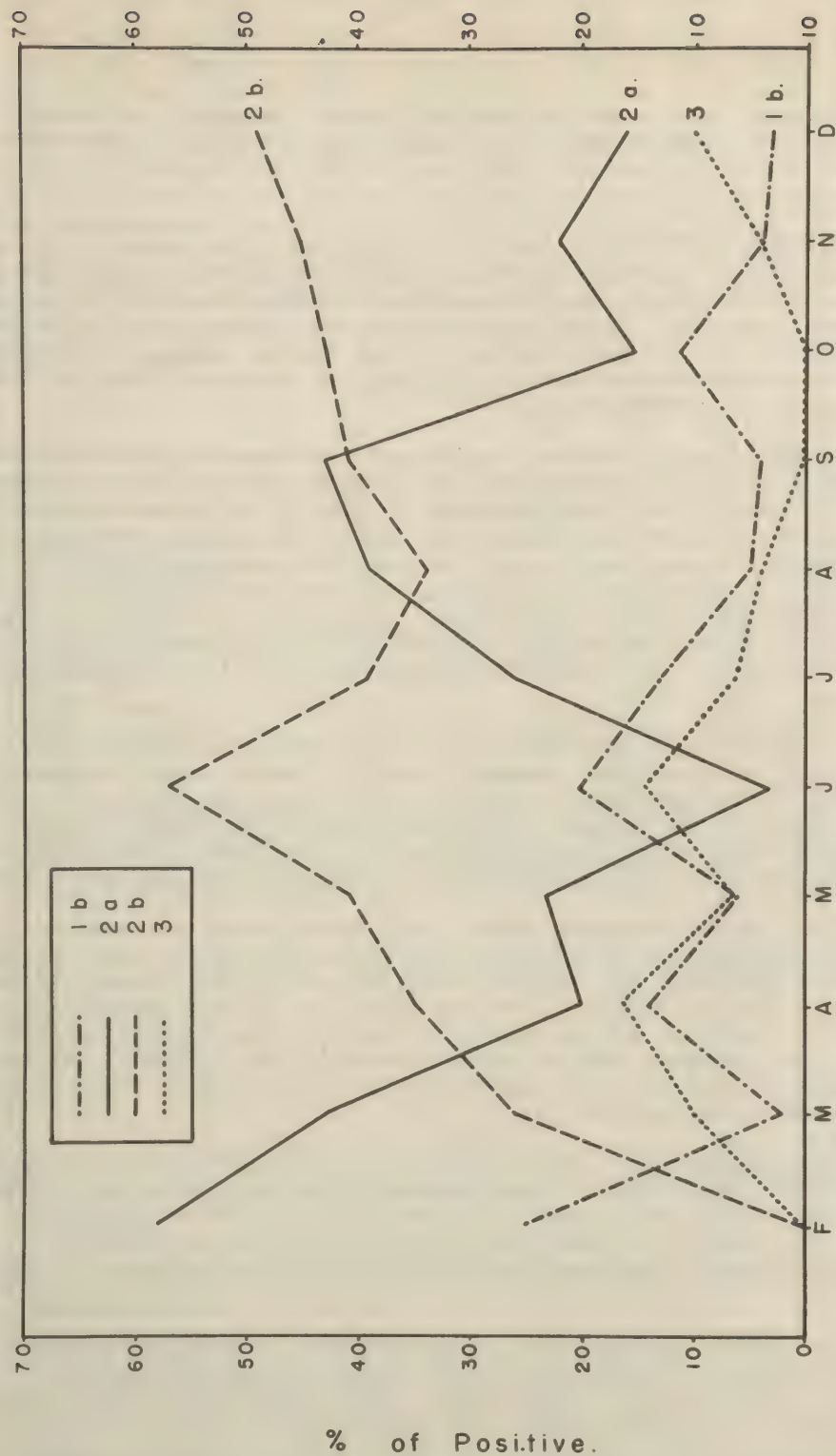


Figure 3. Seasonal Fluctuation in Predominant Sh. flexneri Types in Tokyo, 1952

Table XXXIII. Mannite-Fermentation in Korean Strains of Sh. flexneri 4

<u>Sh. flexneri</u> 4	Jan.	Feb.	Mar.	Apr.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Mannite pos.	185	116	127	26	10	9	5	66	58	8	610
%	100%	100%	92%	79%	46%	29%	72%	76%	74%	42%	85%
Mannite neg.	0	0	11	7	12	22	2	21	21	11	107
%	0%	0%	8%	21%	54%	71%	28%	24%	26%	58%	15%

Non-Specific "X" and "Y" Strains - During 1952, 4 strains from Japanese patients were found to react in an atypical non-specific manner. Agglutination occurred only in "X" and "Y" serums. Biochemical reactions were consistent with the Sh. flexneri pattern. Indol formation varied with colony morphology; translucent colonies were indol-negative; opaque colonies were indol-positive. Thermolabile antigens were absent and group factor "3" was present. No specific factors could be demonstrated despite passage on several media. It is possible that these strains were Sh. flexneri 2b variants, since the 1952 Japanese epidemic strains were predominantly 2b.

Agglutinins in Shigellosis - Although agglutination procedures for Shigellosis have not been found useful as an aid in diagnosis, the antibody response is of inherent interest with relation to specific and group antigenic factors in the causative agent (47). During 1952, the serum of 60 patients was examined for agglutinins for Sh. dysenteriae 1 and 2 (ambigua), flexneri 1a, 1b, 2a, 2b, 3, 4a, 5, 6, "X", "Y", and rabaulensis, Sh. boydii, Sh. sonnei, A-D 1, 2, 3, and 4, and, in each case, the homologous strain isolated from the patient. The homologous strains included Sh. flexneri 1b, 2a, 2b, 3, 4a, Sh. sonnei, and Sh. dysenteriae 2. Repeat serums were obtained at weekly intervals on 42 patients, while single specimens of early convalescent sera were tested on the remaining 18 patients.

Homologous strains and stock antigens reacted to approximately equivalent titers. In the case of Sh. flexneri 2a infections, however, 3 out of 5 cases showed a higher titer (4-fold) in tests with the homologous strain than with the stock strain. Specific type and heterologous titers did not follow a consistent pattern; the highest titers occurred with Sh. flexneri variant "Y", rabaulensis, and A-D 3 antigens. The latter observation was unexpected, and may indicate presence of coliform agglutinins in the serum.

The results with type specific antigens showed that the major antibody response in Shigellosis caused by Sh. flexneri is associated with group factors rather than specific factors. The level of response with type specific antigens was slightly lower than has been previously reported. This is believed to be due to the use of test suspensions prepared from antigenically complete strains with group factors possibly less accessible than was the case with stock antigens used by earlier investigators. Since it was assumed that the virulence of Shigella infections is associated with type-specific factors, this response to group antigens was unexpected.

Atypical Shigella dysenteriae - A large group of organisms which reacted with Shigella flexneri grouping serum, but not with type-specific serum, constituted a major problem in identification. These strains were referred to in 1951 (7) as possessing antigenic factors in common with the A-D and the Sh. dysenteriae groups. Detailed classification studies, including cross-reactions and absorptions are shown for 3 representative strains.

Strains 891, 2499, and 3162, isolated from dysentery cases in Korea, were nonmotile, gram-negative rods, forming indol and fermenting glucose, trehalose, maltose, arabinose, xylose, and sorbitol with acid but no gas. Thirteen additional sugars were not fermented. The strains were urease-negative and did not utilize citrate as a sole source of carbon. Principal serologic reactions in addition to Sh. flexneri, occurred with Sh. dysenteriae 1 and A-D 1. A cross-relationship between the A-D group and the Sh. dysenteriae has long been known, however, fermentative reactions of these organisms

differentiate these strains. Using both heated and unheated antigens antisera were prepared and tube agglutination tests done with cross-reactions as shown in Table XXXIV. The cross-reactions indicated strains 891 and 2499 to be closely related. Strain 3162 displayed a thermolabile blocking antigen, which when removed by heating allowed significant cross-reactions with 891 and 2499. Each strain showed weakly antigenic thermolabile blocking antigens. These factors were not uniform in structure, since unheated 2499 agglutinated to titer in 891 and 3162 sera of both types, while 891, unheated, did not. Strain 3162 had a blocking antigen which was reactive with the heterologous as well as with homologous sera. Cross-reactions with Sh. dysenteriae were strong with 891 and 3162, but weak with 2499. Antibody to the latter, prepared with heated antigen, did not react with Sh. dysenteriae.

Table XXXIV. Serologic Cross-Reaction of Strains 891, 2499, and 3162 in Terms of Reciprocal of Highest Positive Dilution

Antigen Serum										
	891 Unheated	891 Heated	2499 Unheated	2499 Heated	3162 Unheated	3162 Heated	<u>Sh. dys. 1</u> Unheated	<u>Sh. dys. 1</u> Heated	A-D 1, un- heated	A-D 1, Heated
891 Unheated	80	5120	5120	5120	160	640	1280	2560	5120	5120
891 Heated	-	5120	5120	5120	160	5120	40	40	5120	5120
2499 Unheated	160	5120	5120	5120	320	1280	160	320	40	2560
2499 Heated	-	5120	5120	5120	80	320	-	-	2560	2560
3162 Unheated	40	5120	5120	5120	2560	5120	320	1280	2560	2560
3162 Heated	40	5120	320	5120	5120	5120	80	80	2560	2560

Heterologous absorption (Tables XXV, XXVI) of 891, or of 3162 antiserum by one of the three antigens heated resulted in removal of Sh. dysenteriae 1 and most of A-D 1 antibody. Unheated antigen failed to absorb A-D 1 antibody. The heterologous reactions indicated strains 2499 and 3162 differ in some antigenic factors.

Table XXXV. Titers of the Serum Prepared Against 891 Unheated

Test Antigen Absorbing Antigen										
	891 Un- heated	891 Heated	2499 Un- heated	2499 Heated	3162 Un- heated	3162 Heated	<u>Sh. dys. 1</u> Unheated	<u>Sh. dys. 1</u> Heated	A - D 1, un- heated	A - D 1, heated
Unabsorbed serum	80	5120	5120	5120	160	640	1280	2560	5120	5120
Absorbed with:										
<u>Sh. dysenteriae</u> 1, unheated	5120	5120	5120	5120	160	640	-	-	2560	5120
A - D 1, heated	5120	2560	1280	2560	320	5120	-	2560	-	-
<u>Sh. dys. 1</u> and A - D 1 heated	80	1280	2560	1280	160	640	-	-	-	-
2499 unheated	80	320	-	-	80	-	-	-	160	160
2499 heated	-	40	-	-	-	-	-	-	-	-
3162 unheated	160	160	160	40	-	-	-	-	160	40
3162 heated	80	320	320	160	-	-	-	-	-	-

Table XXXVI. Titer of Serum Prepared Against 3162 Unheated

Absorbing Antigen	Test Antigen									
	3162 Un-heated	3162 Heated	891 Un-heated	891 Heated	2499 Un-heated	2499 Heated	Sh. dys. 1 unheated	Sh. dys. 1 heated	A - D 1, un-heated	A - D 1, heated
Unabsorbed	2560	5120	40	5120	5120	5120	320	1280	2560	2560
Absorbed with: Sh. dysenteriae 1 unheated	5120	5120	-	2560	1280	5120	-	-	2560	2560
A - D 1, heated	1280	1280	40	1280	2560	1280	40	1280	-	-
Sh. dys. 1 and A - D 1 heated	40	640	40	640	320	1280	-	-	-	-
891 unheated	-	40	-	-	-	-	-	-	-	-
891 heated	-	-	-	-	-	-	-	-	-	-
2499 unheated	80	80	640	-	-	-	-	-	-	-
2499 heated	-	-	-	-	-	-	-	-	-	-

The unknown organisms are closely related to both Sh. dysenteriae 1 and A-D 1. They are not identical with either species, and in that respect are consistent with their behavior biochemically, wherein they are also intermediate between the two strains. Until further information can be obtained, the strains have been designated as representative of a large group of *Shigella*-like organisms intermediate taxonomically between Sh. dysenteriae 1 and A-D-1.

Antibiotic Sensitivity of *Shigella* Strains from Japan and Korea - Periodic checks of antibiotic sensitivity of the principal *Shigella* types occurring in the Far East were made to detect the appearance or rise in incidence of resistant strains. In March, 130 strains and in December 150 strains from both Japan and Korea were tested for antibiotic sensitivity. Antibiotic was incorporated in agar plates in graded dilutions, and seeded with freshly isolated cultures. Absence of visible growth was used as the endpoint of sensitivity. Table XXXVII summarizes the results.

Table XXXVII. Geometric Mean Value of Inhibiting Levels of Antibiotic for Fresh *Shigella* Strains

Organism	No. Tested	Chloromycetin mcg/ml	Aureomycin mcg/ml	Streptomycin mcg/ml	Terramycin mcg/ml
Sh. dysenteriae 1	3	2.5	15	6.2	3.7
Sh. dysenteriae 2	14	2.5	10.7	9.5	3.3
Sh. flexneri 1a	22	2.5	14	8.5	3.5
Sh. flexneri 2a	52	2.5	14	8.9	3.5
Sh. flexneri 2b	14	2.7	13	7.0	3.5
Sh. flexneri 3	18	2.6	13	7.7	3.5
Sh. flexneri 4	75	3	14	8.2	3.5
Sh. flexneri 5	73	2.6	14	8.5	3.5
Sh. flexneri "X"	1	3.8	15	7.5	2.5
Sh. flexneri "Y"	8	2.5	13	8.3	3.3

The differences observed between the March and December readings were not significant. A slight increase in resistance to streptomycin and terramycin was noted in

December. The sensitivity to chloromycetin and streptomycin was relatively unaltered in a comparison with results of tests in 1951, but a definite increase in resistance to aureomycin and terramycin occurred during that time. In 1951, 34 strains of *Shigella* were inhibited at from 5 to 10 mcg. of aureomycin per ml. of medium; in 1952, the geometric mean values varied from 11 to 15. Terramycin in 1951 was inhibitory for Korean *Shigella* in concentrations averaging 2.5 mcg/ml; the mean inhibiting value in 1952 was 3.5 mcg/ml.

PHASE VARIATION IN SHIGELLA FLEXNERI 4: The phenomenon of phase variation in *Shigella* was first described in detail by Boyd (49) in 1938. It has usually been alluded to as dissociation due to prolonged culture on artificial medium, not of importance in affecting the results of serologic typing of *Shigella*, not widely observed. Experience with the *Sh. flexneri* 4 epidemic referred to earlier in this report showed that phase variation occurs very quickly, if not in fact in the patient.

In 1951, phase B was first recognized in a strain of *Sh. flexneri* 4 from Korea, when colonies being checked for identity failed to agglutinate in any but *flexneri* "Y" serum*. Additional specific colonies of strains of *flexneri* 4 were plated from fresh isolates and subcultures were checked for phase. Less than 5 percent of the colonies reacted in "Y" serum.

Five to ten strains from each lot of cultures were plated on MacConkey's agar. Ten to 20 colonies were subcultured on tryptose agar slants, and each slant culture typed with specific and "Y" serum. Three types of reaction occurred: agglutination in "Y" serum only, in type-specific 4 only, and in both type-specific 4 and "Y". Subcultures of those strains which agglutinated in A and B serum repeatedly yielded A or B phase colonies and occasional AB reactors, but no pure AB strain was ever secured. It was concluded that all specific reacting colonies, regardless of whether or not they reacted in non-specific serum as well, should be classified as "specific phase"; non-specific applies only to strains failing to react in phase A. A true biphasic strain - i.e., one that on continued subculture reacts as an AB, was not found among the several thousand strains tested. Contrary to reports of Wheeler and of Boyd, no morphologic differences between A and B phase colonies were seen. Variants occurred, but they were not related to phase.

Incidence of Phase Variation in Fresh Isolates of *Shigella flexneri* 4 - Nineteen lots totalling 174 strains of *Sh. flexneri* 4 from Korea were examined between February and August, 1951. Strains were received on original KIA slants, plated to MacConkey's agar, and then to slants. The number of strains in which phase "B" occurred was determined, together with the total number of "B" and "A" colonies recovered. Table XXXVIII summarizes the findings and illustrates fluctuations occurring in the *Sh. flexneri* 4 population. Initial observations revealed up to 48% of colonies in phase B in one group of cultures, and over 80% of strains showing dissociation on first subculture. The parallel between the number of strains yielding phase "B" colonies and the percentage of phase "B" colonies is consistent with the assumption that dissociation is a genetic function. The incidence of dissociation fell as summer progressed. The dissociation rate for the season was 10.0% of total colonies examined. Of these strains 44.2% were in the specific phase.

One hundred four strains from Korea were tested in groups of 5 during the period from May to December. Again 10 colonies were picked for each strain when the culture was plated from its initial KIA slant. Seventy (67%) reacted only in the specific phase, and 34 (23%) yielded phase "B" as well as phase "A" colonies. A significant drop in the number of strains growing in both phases was noted, in contrast to those found in 1951. The percentage of pure phase "B" colonies was correspondingly reduced (4.2% in contrast to 10.0% noted in 1951).

* "Y" was at this time prepared from nonspecific 4a, absorbed with "X".

Table XXXVIII. Phase Dissociation in Freshly Isolated Strains of Sh. flexneri 4 from Korea During February to September, 1951

Lot No.	Total Strains	Colonies Checked per Strain	Total Colonies Checked	Total Phase "B" Colonies	% of Pure Phase "B" Colonies	Total Strains Showing Phase "B"	% of Total Strains Showing Phase "B"
1	17	10	170	9	5.2%	8	47%
2	17	10	170	9	5.2%	8	47%
3	5	20	100	18	18.0%	2	40%
4	5	10	50	24	48.0%	5	100%
5	10	20	200	41	20.5%	8	80%
6	9	20	180	53	30.0%	7	77%
7	10	20	200	8	5.0%	5	50%
8	8	20	160	20	12.5%	4	50%
9	9	20	180	26	14.4%	7	77%
10	9	20	180	10	5.5%	5	55%
11	9	20	180	16	8.8%	3	33%
12	12	10	120	4	3.3%	4	33%
13	5	10	50	7	14.0%	2	40%
14	6	10	60	4	6.6%	2	33%
15	6	10	60	4	6.6%	2	33%
16	8	10	80	2	2.5%	2	25%
17	19	20	380	8	2.1%	2	10.5%
18	4	10	40	1	2.5%	1	25%
19	6	20	120	4	3.3%	1	16.6%
Total 174			2540	268	Ave. 10.0%	78	Ave. 45.8%
and Ave.							

A qualitative difference between Korean and Japanese strains of Sh. flexneri was noted, in that 10 strains from Japanese, subcultured 20 generations with test of each subculture, for a total of 2,000 colonies, showed no dissociation. Subsequent strains obtained in Japan were also monophasic. This distinction is a striking example of regional variation among Shigella types. The monophasic Japanese strains were mannite-negative, while the Korean strains fermented mannite.

In addition to establishment of the incidence of phase variation on primary culture, a small series of cultures was carried through successive generations with subculture in specific, mixed, and non-specific phases. The specific phase was recovered from mixed and, rarely, from seemingly completely non-specific strains. It is assumed, in keeping with Boyd's hypothesis, that these "non-specific" strains harbored undetected specific elements. In a certain percentage of colonies, non-specific variants appeared, but the dissociation rate appeared to reach a maximum level, usually at between 25% and 30% of the colonies picked. No authentic instance of reversion from pure phase B to phase A was noted among all Sh. flexneri 4 strains studied.

Phase variation in other types, notably Sh. flexneri 2, 3, 3-X, 1-Y, and 1-3, was noted in small numbers of strains during this period. The mechanisms controlling these variations may differ qualitatively from those observed for Sh. flexneri 4a.

Sh. flexneri 4 Phase Variation and Mannite Fermentation - The major part of this study was carried out from September to December, 1952, during which time the first mannite-negative strains from Korea were being studied, and a comparison of phase behavior of mannite-fermenting and non-fermenting strains from Korea was made. Table XXXIX summarizes the results of observations over a 4 month period.

Out of the 81 strains tested for phase relationship during this 4-month period, 49 were mannite positive, and 32 were mannite-negative. Sixty nine of the 81 strains

were in the specific phase. Of these, 41 were from mannite-positive strains, while 28 were from mannite-negative strains. This seems to indicate a preponderance of specific phase strains among the non-mannite fermenters.

Table XXXIX. Sh. flexneri 4 Phase Variation in Relation to Mannite-fermentation: Korea, 1952

	Sept.		Oct.		Nov.		Dec.		Total	
	No.	% of Total	No.	% of Total	No.	% of Total	No.	% of Total	No.	% of Total
Total Strains Received	7		87		79		19		192	
Mannite-positive	5	72%	66	75.8%	58	74.6%	8	42.1%	137	71.3%
Mannite-negative	2	28%	21	24.2%	21	24.4%	11	57.9%	55	28.7%
Strains Tested for Phase	5	% of Total Tested	37	% of Total Tested	30	% of Total Tested	9	% of Total Tested	81	% of Total Tested
Total Specific	2	40%	35	94.5%	25	83.3%	7	77%	69	85.1%
Total Non-specific	3	60%	2	5.5%	5	16.7%	2	33%	12	14.9%
Mannite-Positive Strains										
Total	4		27		15		3		49	
Specific Phase	2	50%	25	92.5%	13	86.6%	1	33.3%	41	83.6%
Non-specific Phase	2	50%	2	7.5%	2	13.4%	2	66.6%	8	16.4%
Mannite-Negative Strains										
Total	1	% of Total	10	% of Total	15	% of Total	6	% of Total	32	% of Total
Specific Phase	0	-	10	100%	12	80%	6	100%	28	87.5%
Non-specific Phase	1	-	0		3	20%	0		4	12.5%

Phase Variation in Successive Generations of Sh. flexneri 4 - A study was made of phase behavior during serial passage of 35 strains, including 21 which had been held in semisolid tryptose-phosphate agar under sterile mineral oil at room temperature for several weeks, and 14 taken from KIA slants at time of primary isolation. The effect of serial passage on phase with reference to mannite fermentation is summarized in Table XL.

Table XL. Phase Variation and Repeated Subcultures of Sh. flexneri 4

No. of Strains	No. of generations	Mannite-Positive			Mannite-Negative		
		Specific Only	Biphasic	Non-specific Only	Specific Only	Biphasic	Non-Specific
6	18	2	4	0			
3	9	0	0	3			
15	5	0	15	0			
9	10				9	0	0
2	5				2	0	0
35		2	19	3	11		

Total colonies checked for phase variation: 5104
 No. colonies in specific phase: 4602 (90.1%)
 No. colonies in non-specific phase: 502 (9.9%)

Thirty strains from Korea, 4 from Ryukyus, and 1 from Japan were included. Of the mannite-positive strains, 20 were from stock cultures and four were fresh isolates, all

from Korea. Of the mannite-negatives, 10 were fresh isolates and one from stock culture. Of these 6 were from Korea, 4 from Okinawa, and one from Japan.

Of 13 strains in the specific phase only for 5 to 18 generations, 11 were mannite-negative. None of the mannite-negative strains yielded non-specific phase organisms even after being held in stock culture, in contrast to the 4 biphasic mannite-negative strains shown in Table XXXIX. All but two mannite-positive cultures split off non-specific colonies. The two which did not were fresh isolates. Two other fresh mannite-positive isolates were consistently biphasic. Of 502 non-specific phase colonies, 161 were recovered in the first plating of the strain from stock culture. There was no increase in proportion of non-specific colonies on serial passage of these strains.

Discussion - Phase variation is a consistent characteristic of the mannite-positive Shigella flexneri 4 Korean strains. Approximately half of the initial isolates showed biphasic or phase "B" as well as phase "A" colonies. After 10 to 18 subcultures the biphasic strains increased to 90%. A small group of monophasic specific mannite-positive cultures persisted as such despite repeated transfers. The mannite-negative strains did not dissociate during serial transfer, with the exception of 4 strains encountered during the last 4 months of 1952 in Korea. These were the first such strains observed in the Far East.

From the practical aspect, phase variation in Shigella flexneri 4 is important in that it offers a constant source of error in serologic typing because of the great variation in cross reactions of the non-specific phase. The non-specific variant is less virulent for mice but slightly more resistant to antibiotics (7). Strains in the specific phase are customarily expected in dysentery infections. Our results indicate that the non-specific phase may occur in fresh isolates along with the specific phase.

THE GROUP FACTORS OF SHIGELLA FLEXNERI 4 - The confused early history of serologic study of Shigella due to lack of understanding of group and specific factors, was clarified by Boyd's concept of type and group-antigens in the flexneri group. Weil, Farsetta and Knaub confirmed this observation (50). Wheeler (51) proposed a total of 9 group factors to characterize Sh. flexneri in addition to type specific factors. Of these, "1" is supposedly present in all strains while 7, 8, and 9 are a closely linked set of factors characterizing Sh. flexneri 3.

During 1951 and 1952 it became increasingly evident that use of type specific antigens did not effectively differentiate epidemiologically distinct strains of Shigella. Group factor antisera of the flexneri series were therefore prepared following chiefly the interpretation of Wheeler and of Madsen (52).

- (1) Factor "3": Antiserum for Shigella flexneri "Y", a non-specific phase of Sh. flexneri 2a (Strain ES-72) was absorbed with Sh. flexneri "Y", EW-1678, a variant lacking factor "3".
- (2) Factor "3" and "4" (mixed): The original serum was the same as used for factor "3", but when absorbed by Sh. flexneri "Y" EW-1677, factors "3" and "4" both remain. This serum is the complete non-specific phase for study of phase B for flexneri 4.
- (3) Factor "4", "12": A provisional new group factor is proposed (see below). Sh. rabaulensis antiserum, prepared against a strain from the Army Medical Service Graduate School was absorbed with Sh. flexneri "Y" ES 827-B, a non-specific phase of Sh. flexneri 4a. Despite painstaking attempts, no pure factor "4" has been demonstrable up to this time. Factor "4" must then be determined by "3, 4," and "4, 12" antisera.

Five different strains of "Y" variant and Sh. rabaulensis were used in attempts to prepare pure group "4" antibody. Strains EW-1678 (Army Medical School) and rabaulensis (Army Medical School) contain factor "4" plus additional distinct provisional factors.

Strains ES-388, ES-72, and ES-129B each contain at least "3" and "4". In each instance, cross-absorption to produce a pure group "4" antibody resulted in absorption of group factor "4" or persistence of the provisional factor "12". The conclusion was reached that factor "4" was incompletely characterized by Boyd, Wheeler, and others who have reported on its use.

- (4) Factor "6": Antiserum for Sh. flexneri 1b, strain ES-53, which was isolated in this laboratory in 1949 from a Japanese, was absorbed with Sh. flexneri variant "Y" ES-129B, and by Sh. flexneri 1a Army Medical School.
- (5) Factor "X" ("7", "8", and "9"): This complex is not usually separated, and in fact the three factors are closely related. An antiserum for Sh. flexneri "X", EW-1678, was absorbed with Sh. flexneri "Y" to produce the series of factors.

Provisional New Group Factors - Wheeler and Madsen each indicated that not all possible factors were characterized in their descriptions of group factors. During 14 months of investigation of Sh. flexneri types, with emphasis chiefly on type 4, antigenic differences associated with differences in mannite fermentation were noted. A series of additional factors were derived and designated in the same manner as those of Boyd and of Wheeler.

- (1) Provisional group factor "10": Antiserum for a non-specific phase of Sh. flexneri 4a, isolated here from a Japanese in 1949 (strain 129-B) was absorbed with Sh. flexneri "Y" strain ES-72.
- (2) Provisional group factor "11": Antiserum for Sh. flexneri variant "Y", 827B, a non-specific phase of 4a from CDC, was absorbed with Sh. flexneri "Y", 129-B.
- (3) Provisional group factor "12": Antiserum for Sh. rabaulensis, Army Medical School, was absorbed with Sh. flexneri variant "Y", strains 129-B (13) and 827B. Although factors "4" and "12" have not been successfully separated to yield "4", the converse manipulation was possible.
- (4) Provisional group factor "13": Antiserum of Sh. flexneri variant "Y" strain 1678, absorbed with Sh. flexneri variant "Y" 129B.
- (5) Provisional factor "14": Antiserum of Sh. flexneri "Y" Army Medical School absorbed with Sh. flexneri variant "Y", 129B.

The fact that different reproducible factors can be regularly derived from strains of Sh. flexneri 4 phase B indicates that although they are heterogeneous, type 4 organisms can be segregated in a systematic fashion by detailed group factor analysis.

Differentiation of Sub-Types of Sh. flexneri 4 - Sh. flexneri 4a, as described by Boyd, Wheeler, Madsen, Ewing (53) and others, contains type specific factor 4 in the specific phase and no specific antigen in the non-specific phase. This type was described as mannite-fermenting (54) with occasional exceptions noted by Ewing. Sh. rabaulensis (Sh. rio of Weil et al) (55) was described by Madsen as a mannite-negative type 4, containing group factor "3", and probably equivalent to type 4a. The Subcommittee on Nomenclature of the Enterobacteriaceae, however, accepted it as Sh. flexneri 4c, and it is generally so designated. Positive xylose-fermentation distinguishes this type from Sh. flexneri 4a.

Sh. flexneri 4b is characterized by group factor 6. It has not been observed in over 2100 strains of Sh. flexneri 4, each of which was checked for group factor "6".

When it became apparent that variation in mannite fermentation permitted designation of major categories of Sh. flexneri 4 not consistent with the current differentiation of types 4a and 4c, and when differences in phase behavior between mannite-

positive and mannite-negative strains were observed, group factors were determined. The factors described above were applied to a large series of freshly isolated *Sh. flexneri* 4 strains. A definite taxonomic arrangement was evolved. The categories recognized are summarized in Table XLI.

Table XLI. Antigenic Structure and Reaction Patterns of *Sh. flexneri* 4

Designation	Found In	Mannite	Phases Occurring	Group Factors
"J"	Japan	Negative	Specific Non-specific	(3)(4) - - - - - 3, 4, 10 - - - - -
	Korea	Positive	Specific Non-specific	3,(4) - - - - - 3, 4, 10 - - - - -
"R"	Korea, Ryukyus, Japan	Negative	Specific	- - - 11, - - -
			Non-specific	- 4, - 11, 12, - -
"C"	("4c" of CDC) Korea	Positive	Specific	3, 4, - -- 12, - -
		or Negative	Non-specific	3, 4, 10, - 12, - -
"P"	Philippines, Korea, Ryukyus	Positive or Negative	Specific	3, 4,(10) - (12)(13) 14

() = factor sometimes absent in this phase.

Subtype "J" is almost exclusively found in Japan and is mannite-negative. An antigenically similar form found in Korea is mannite-positive. The specific phase may fail to react with any group factors or may react with factor "3". In what has been designated intermediate form, factor 4 appears. The non-specific phase shows "3", "4", and "10" factors.

Subtype "R" is always mannite-negative. The specific phase shows factor "11". Factors "4" and "12", in the non-specific phase, are distinctive. It was first recognized in cultures from Korea in 1952; since then it has been found in the Ryukyus and in Japan. The non-specific phase of a stock strain of *Sh. rabaulensis* contained these same group factors. For this reason it appears that *Sh. rabaulensis* is actually a variant of *Sh. flexneri* 4a.

Subtype "C" has factor "12", but lacks factor "11". Mannite may or may not be fermented. The *Shigella* described as 4c by the Committee on Nomenclature probably belongs here.

Subtype "P" is characterized by its possession of Factors "13" and "14". Other group factors are usually demonstrable. Only the specific form has been observed thus far, and dissociation does not occur. This sub-type was first recognized in Korea. Factor "14" is always demonstrable in *Sh. flexneri* 2a, which fact has finally explained numerous discrepancies in identification attempted with incompletely absorbed specific serum. Thus *Sh. flexneri* 2a would be identified as *Sh. flexneri* 4 due to cross-reaction in type-specific serum.

The distribution of sub-types according to the patterns described is as follows:

Total Strains	216
Subtype "J" (factors 3,4,10)	92
Subtype "R" (factors 4,11,12)	95
Subtype "C" (factors 3,4,10,12)	9
Subtype "P" (factors 3,4,10,12,13,14)	20

Group Factors and Phase Variation in *Sh. flexneri* 4 - The phase variation was reviewed in terms of group factors. Mannite-positive strains from Korea were shown to exhibit factor "3" in the specific, and "3", "4", "10" in the non-specific phase. When colonies showing only factor 3 were plated in successive subcultures, no non-specific phase colonies were found in 8 generations. This finding establishes a relationship between phase variation and group factor content. Subsequent observations indicate that the degree of dissociation varies directly with the intensity of positive reaction to factor "10" antiserum.

Mannite-negative *Sh. flexneri* 4 from Korea, Japan, and Ryukyus all exhibited only factor "11". No reaction in "Y" occurred. After prolonged subcultures, these strains still reacted only with specific serum, indicating that factor "11" is not associated with prompt dissociation. A pure non-specific phase was artificially derived for this strain. It contained factors "4", "11", and "12".

SALMONELLA TYPES IN JAPAN AND KOREA DURING 1952: There were 278 strains of *Salmonella* identified from Korea during 1952, as contrasted with 937 strains identified in 1951. These strains were chiefly from stool cultures; however, 37 strains from blood cultures were received, all from Korea. In Japan, 25 strains were recovered from Americans and 3 were isolated from Japanese nationals.

Stools and rectal swabs were plated on SS agar, and appropriate colonies transferred to Kligler's Iron Agar. In Korea, Kauffmann's modification of tetrathionate broth was effective in enrichment cultures. Organisms positive for indol and/or urease, and those fermenting lactose, sucrose or salicin were discarded. Motility was determined and phases separated in semisolid tryptose agar. Polyvalent "O" *Salmonella* serum was used to select probable strains, and complete typing was performed using the methods of Kauffmann (56) and Edwards and Bruner (57). Resume of findings is presented in Table XLII.

The rate of *Salmonella* infection in Japan, among Americans and among Japanese, was noticeably lower than in 1951. Of 25 strains recovered from Americans 12 were *Sal. typhimurium*. Among strains usually regarded as potential causes of enteric fever, only *Sal. paratyphi* A was encountered. Types not previously recorded from Americans in Japan included *Sal. brandenburg*, *thompson*, *puerto-rico*, *glostrup*, *meleagridis*, and *welt-evreden*. No Group D strains were found and no case of typhoid fever in an American reached the attention of this laboratory. It is interesting to note that with the relatively sparse *Salmonella* flora observed, the 11 types noted include 6 which did not appear in Korea.

The Korean strains showed a predominance of Group D, with *Sal. enteritidis* and *Sal. blegdam* most important. The latter type was the principal agent in the enteric epidemic in the POW camps, and has never before been reported in a large epidemic. Of the *Sal. typhi* strains, 11 were Vi-positive and 5 were Vi-negative. *Paratyphi* A was numerically important and appeared with explosive abruptness at intervals. The relationship of *Sal. paratyphi* C and of *Sal. choleraesuis* is of taxonomic and epidemiologic interest. *Sal. paratyphi* C strains are known to vary in behavior with regard to phase, H₂S formation, and fermentation of trehalose and arabinose. The relationship of the strains is shown in Table XLIII.

The predominant form of *Sal. paratyphi* C was the H₂S, trehalose, and arabinose positive. Six of the H₂S positive and 2 of H₂S negative strains fermented only one of the two sugars. No strains were negative in both sugars.

Table XLIII. Incidence of Salmonella Types by Geographic Area

Group		Japan		Korea	
		Americans		Japanese	
		No.	%	No.	%
A	Sal. paratyphi A			2	66
				65	23.4
B	Sal. paratyphi B (Java)			1	0.4
	Sal. paratyphi B	2*	8.0	18	6.5
	Sal. san diego	1	4.0		
	Sal. typhimurium	12	48.0	9	3.3
C ₁	Sal. paratyphi C Vi /			10	3.3
	Sal. paratyphi C Vi -			29	10.4
	Sal. choleraesuis			1	0.4
	Sal. branderup	2*	8.0		
	Sal. montevideo	1	4.0	4	1.4
	Sal. thompson	1	4.0		
	Sal. oranienburg	2	8.0	7	2.5
	Sal. bareilly			3	1.1
	Sal. tennessee			1	0.4
C ₂	Sal. muenchen	1	4.0		
	Sal. puerto rico			1	0.4
	Sal. glostrup	1	4.0		
D	Sal. typhosa Vi /			11	3.9
	Sal. typhosa Vi -			5	1.8
	Sal. enteritidis			49	17.7
	Sal. blegdam			58	20.9
	Sal. pullorum			1	0.4
E ₁	Sal. meleagridis	1	4.0		
	Sal. weltevreden	1	4.0		
E ₃	Sal. senftenberg			2	0.7
G	Sal. atlanta			2	0.7
	Sal. worthington			1	0.4
Total		305	25	3	278

* One strain recovered from an American on Okinawa

EVALUATION OF SHIGELLA TYPING SERUM: At the request of the Immunology Division, AMSGS, Shigella typing serum produced by the Communicable Disease Center of the U.S. Public Health Service was evaluated. The sera were not as highly absorbed as those currently being issued by AMSGS, and cultures of Shigella and the Alkalescens-Dispar Groups were provided for use in further absorption if this should be indicated.

Table XLIII. Phase and Cultural Patterns of Sal. paratyphi C and Choleraesuis

Type	No. Of Strains	Phase	H ₂ S	Trehalose	Arabinose	Vi /	Vi -
<u>Sal. paratyphi C</u>	25	Diphasic	Positive	Positive	Positive	7	18
<u>Sal. paratyphi C</u>	1	Diphasic	Positive	Positive	Negative	0	1
<u>Sal. paratyphi C</u>	5	Diphasic	Positive	Negative	Positive	1	4
<u>Sal. paratyphi C</u>	6	Diphasic	Negative	Positive	Positive	3	3
<u>Sal. paratyphi C</u>	1	Diphasic	Negative	Positive	Negative	0	1
<u>Sal. paratyphi C</u>	1	Diphasic	Negative	Negative	Positive	1	0
<u>Sal. choleraesuis</u> var Kunzendorf	1	Diphasic	Positive	Negative	Negative	-	-

Sera from CDC, the AMGS, and this department were compared, using chiefly the slide agglutination technique. After comparing the sera with stock strain antigens, they were tested on a series of 241 recently isolated strains of *Shigella*. Since thermolabile non-specific blocking antigens have frequently been encountered in *Shigella* in the Far East, this factor was taken into account by testing a series of antigens before and after heating in boiling water bath for 20 minutes (58), (59).

Sh. dysenteriae sera, types 1 through 7, were tested with stock strains of all types of *Sh. dysenteriae*, *flexneri*, *boydii*, *sonnei*, and A-D 1-4. With antigens of *Sh. dysenteriae* 1 through 7, reactions were essentially specific with *Sh. dysenteriae* sera from all 3 sources. When *Sh. dysenteriae* sera were tested against stock strains of the *flexneri* and *boydii* groups, AMGS and 406th sera showed no reactions with any *flexneri* types, but among the CDC sera, cross-reactions occurred with *Sh. flexneri* 1a, 1b, 4b, 5 and "Y", in some cases with unheated, and in other cases with heated antigens. *Sh. boydii* stock strains of types 3, 4, 5, 6 and 7 reacted with CDC but not with AMGS or 406th *dysenteriae* antisera. Similar cross-reactions occurred with the A-D group antigens in the CDC *dysenteriae* sera. Thermolabile blocking antigens were present in the A-D group test strains. *Sh. flexneri* type sera, tested with the complete spectrum of *Shigella* species and types, showed the most extensive cross-reactions. CDC 4a serum reacted with antigens of *Sh. dysenteriae* types, while AMGS and 406th did not. Among the *flexneri* strains, CDC *Sh. flexneri* sera crossed with all *flexneri* types to an extent that made specific identification impossible. Both AMGS and 406th sera were specific for *flexneri* types.

Sh. boydii sera were in substantial agreement from all 3 sources, as were *Sh. sonnei* sera. A-D sera for groups 1 through 4 reacted specifically in the case of AMGS and 406th MGL, but a wide spectrum of cross-reactions was noted with CDC sera.

Tests with 281 freshly isolated strains included *Sh. flexneri* 1a (16 strains), 1b (3 strains), 2a (51 strains), 2b (7 strains), 3 (70 strains), 4 (71 strains), 5 (40 strains), *Sh. dysenteriae* 1 (7 strains), *Sh. dysenteriae* 2 (3 strains), *Sh. flexneri* variant "Y" (2 strains), *Sh. boydii* 1 (2 strains), 5 (3 strains), 6 (1 strain), and *Sh. sonnei* (5 strains). Results were comparable to those obtained with stock strains. 406th sera yielded virtually specific reactions; AMGS sera crossed with several *Sh. flexneri* strains and in some instances failed to react with heated antigens. CDC *Sh. flexneri* sera were almost completely heterogeneous, and reacted with the whole spectrum of *Sh. flexneri* types. With *Sh. dysenteriae*, *boydii* and *sonnei* sera, reactions were specific for all sources.

Effective antisera could be derived from the CDC sera, after 3 absorptions. It is probable that group factors must receive particular attention if a specific, usable test *Shigella flexneri* serum is to be obtained.

IN VITRO GROUP A STREPTOCOCCUS VARIATION ASSOCIATED WITH STREPTOLYSIN PRODUCTION: Variation of colony morphology of streptococci has been described with relation to type-specific substance, virulence, and toxin (60). However, the correlation of colony variation, cellular morphologic variation, and activity of streptolysin formation in derivatives of a single strain has apparently not been previously described.

Todd noted that certain group A strains, on continued subculture, gradually lost the ability to produce streptolysin "O", but retained the ability to produce streptolysin "S". Colony morphology did not, apparently, change during this progression.

A strain of hemolytic streptococcus, 8668, acquired from the NRRL, showed two morphologic variants on plate cultures. Five percent of the colonies (designated 8668-L) were mucoid, smooth, from 0.5 to 3.0 mm. in diameter, and after 48 hours, showed a raised unbonate center. The remaining colonies (designated 8668-S) were smooth, domed, and from 0.2 mm. to 1.0 mm. in diameter. The 8668-L variants could be consistently derived from the 8668-S form, but the reverse variation under the same conditions was never observed. Microscopically the 8668-L cells were uniform in size, gram-positive, and grew in distinctive long chains averaging 13 cells each. The 8668-S cells varied markedly in size, grew in a clumped formation, varied in retention of gram-stain, and grew at most 4-cell chains. The two forms were completely distinctive microscopically. 8668-L formed a uniform suspension in horse serum, in which 8668-S clumped heavily.

When the two variants were tested for ability to form streptolysin "O", after varying periods of incubation in a modified Todd-Hewitt broth, a marked variation in Streptolysin "O" production was noted. Strain H 46-A, previously used for streptolysin production, was used as a control. Strain 8668-S formed hemolysis which was shown to be streptolysin "O" by appropriate neutralization test. The other variant, 8668-L, failed to form streptolysin "O". The reactions are summarized in Table XLIV.

Table XLIV. Streptolysin "O" Production of Streptococcus
#8668

Incubation Period at 37°C.		8668-L	8668-S	H-46-A (Control)
4 hours	Approx. pH	6.0	7.0	7.2
	M.H.D./ml*	0	32**	8
20 hours	Approx. pH	5.6	6.0	6.2
	M.H.D./ml	0	64	16
48 hours	Approx. pH	5.2	5.6	5.6
	M.H.D./ml	0	16	16

* M.H.D. = Minimal Hemolytic Dose

** Reciprocal of highest reacting dilution

Streptolysin "S" was prepared by suspending cells from a 20-hour blood plate culture in inactivated horse serum for 5 hours at 37°C, followed by centrifugation. The supernatant fluid was titrated for streptolysin "S". Strains 8668-L and 8668-S both produced Streptolysin "S" with "L" reaching a titer of 32 MHD per ml. while 8668-S formed 16 MHD per ml. The identity of the hemolysin was ascertained by its failure to neutralize with antistreptolysin "O", indicating it to be streptolysin "S".

Streptolysin "S" was prepared by inoculating medium containing 1% yeast nucleic acid and incubating at 37°C for 20 hours. Hemolytic activity and neutralization tests were performed with the supernatant fluid of the centrifuged broth culture. Both 8668-L and 8668-S strains produced streptolysin "S" active in a dilution of 1.8.

Antibiotic sensitivity of the parent strain 8668-S and variant 8668-L were determined by a cylinder cup method, using blood agar plates. Both forms were inhibited by .26 mcg. of aureomycin, 2.5 mcg. of chloramphenicol, and 50 mcg. of streptomycin. 8668-L was inhibited by 0.025 u/ml. of penicillin, while 8668-S required 0.05 u/ml. for inhibition. Both forms fermented glucose, trehalose, sucrose, and salicin. Lactose, arabinose, raffinose, sorbitol, glycerol, mannitol, inulin, and starch were

unaltered at 21 days. Methylene blue milk 0.1% was not reduced, gelatin was not liquefied, and milk was coagulated but not digested.

LEPTOSPIROSIS IN JAPAN: At the request of Dr. Haniu of Hiroo Hospital in Tokyo, Japan, serum from a patient with a clinical diagnosis of Weil's disease was studied for leptospiral agglutinins. Twelve stock strains of species of leptospira covering a complete spectrum of antigenic components for the genus ⁽⁷⁾ (AMSGS, 1952) were used as antigens. Screening was performed with 1:100 and 1:400 serum dilutions, followed by titration with those antigens reacting at the screening levels. Results are summarized in Table XLV.

Table XLV. Serum Titers in Patient K , Hiro Hospital

Antigen	Titers: Days after Onset of Illness				
	4 days	13 days	20 days	27 days	39 days
<u>L. ballum</u>	Neg.	1:200	1:400	1:1600	1:800
<u>L. icterohemorrhagiae</u>	Neg.	Neg.	Neg.	1:100	1:200
All other (10) strains	Neg.	Neg.	Neg.	Neg.	Neg.

The serologic picture was consistent with a diagnosis of leptospirosis due to L. ballum, which has not previously been reported from Japan (61). Rats trapped in the restaurant which was the probable source of infection were cultured. Two strains of L. icterohemorrhagiae were recovered, but L. ballum was not found.

The only other human case of leptospirosis observed by this laboratory was that of an American soldier whose serum revealed an agglutinin titer rise for L. icterohemorrhagiae from 0 to 1600 in 13 days.

Table XLVI. Agglutinins for Leptospira in Patient S ____.

Antigen	4 days	7 days	13 days	14 days	20 days	22 days	37 days	55 days
<u>L. icterohemorrhagiae</u>	Neg.	400	1600	1600	800	800	400	200
<u>L. canicola</u>	Neg.	100	100	100	100	200	50	25
<u>L. hebdomadis</u> A.	Neg.	100	200	400	200	200	50	25

Tests for leptospiral agglutinations in cases of aseptic meningitis were conducted on sera from files of the Virus Department, in continuation of a project carried on during 1951. Of 43 convalescent sera two, 52-735 and 53-1259, gave positive reactions. The titers of agglutinins with the interval after onset of symptoms are shown in Table XLVII.

The two cases listed here were not clinically diagnosed as leptospirosis. The reactions of 53-1259, however, strongly suggested presence of leptospiral meningitis, which was reported from Okinawa in 1949. Case 52-735 was of questionable significance, but represents a possible case.

Serum titers of these 2 cases varied from Case S ____, which represented a typical response to L. icterohemorrhagiae. The reaction pattern of 52-1259, with its slow titer rise, resembled the response one would expect to observe if the actual infecting agent were some other related species of leptospira, rather than L. icterohemorrhagiae. In case 52-735, the tentative finding of L. akiyami-A infection was implied.

Table XLVII. Agglutinins in Sera of Cases of Aseptic Meningitis

Case No.		Titer and Time Intervals		
		9 days	16 days	26 days
52-1259	<u>L. icterohemorrhagiae</u>	1:100	1:200	1:400
	<u>L. canicola</u>	1:100	1:100	1:100
	<u>L. hebdomadis A</u>	1:100	1:200	1:100
		1st serum	2nd serum	
52-735	<u>L. akiyami A</u>	1:100	1:200	
	<u>L. hebdomadis PI</u>	1:50	1:100	

In cooperation with the Entomology Department, rodent tissue was collected and inoculated into media; 10 strains of leptospira were recovered from the kidneys of 147 rats trapped in the Tokyo and Kofu areas, during the winter and spring. Fragments of kidney tissues were planted in Fletcher's medium, incubated at room temperature or at 32 C. and observed at 10 and 28 days. Species of rats in Tokyo included 31 R. norvegicus and 3 R. rattus. In Kofu, 104 R. norvegicus, 8 R. rattus and 1 Rattus sp. were taken. Five strains were recovered from each area, for a recovery rate of 14.8% in Tokyo, and 4.4% near Kofu. Only R. norvegicus yielded isolates. All of the strains, after adaptation to Schuffner's medium, were identified serologically as L. icterohemorrhagiae. Table XLVIII summarizes the serologic relationships observed in isolates.

Table XLVIII. Agglutination Reactions of Leptospira Recovered From R. norvegicus in Kofu and Tokyo

Strains	Source	Titers in Antisera			
		<u>L. icterohemorrhagiae</u>	<u>L. canicola</u>	<u>L. akiyami A</u>	<u>L. salinem S.V.T</u>
# 32	Kofu	1/25600	1/1600	1/800	1/1600
# 69	Kofu	1/25600	1/1600	1/400	1/1600
# 80	Kofu	1/25600	1/3200	0	1/800
# 94	Kofu	1/12800	1/3200	1/800	1/1600
# 111	Kofu	1/25600	1/800	1/400	1/1600
# J9	Tokyo	1/25600	1/6400	0	1/6400
# FJ11	Tokyo	1/25600	1/3200	0	1/1600
# FJ14	Tokyo	1/25600	1/3200	0	1/3200
# FJ22	Tokyo	1/25600	1/3200	0	1/3200
# FJ29	Tokyo	1/25600	1/1600	1/200	1/800
<u>L. ictero:</u> <u>control 1</u>		1/51200	1/1600	1/400	1/400
<u>L. ictero:</u> <u>control 2</u>		1/51200	1/1600	1/800	1/800

A completely unexpected result of this study was the recognition of two antigenically distinct groups, each of 5 strains of L. icterohemorrhagiae. One group possessed a common factor with L. akiyami A and resembled the classic description of L. icterohemorrhagiae Wijneberg (63). The other variety lacked the akiyami A factor. Table XLVIII shows the geographic variation in distribution, which may prove to be of epidemiologic significance. None of the rat strains gave the cross-reaction with L. hebdomadis A that was conspicuous with patient S ____, which was diagnosed as L. icterohemorrhagiae infection and the infecting agent in that case may have been antigenically distinctive from the rat strains described.

DEPARTMENT OF PATHOLOGY

During 1952, the Department of Pathology continued its investigations of several of the medical problems which were encountered in 1951. Studies on hemorrhagic fever, infectious hepatitis, complications of battle trauma, including lower nephron nephrosis, gas gangrene and fat embolization, were continued and additional data obtained. Preliminary morphologic investigations on the fate of dextran given to fatal battle casualties were begun. Some of the problems encountered in significant proportions in 1951 were not encountered at all or to a considerably less degree in 1952. These included smallpox, frostbite, enteric disease, and relapsing fever. The volume of routine work increased slightly.

Wherever possible, attempts have been made in this report to include data which could be compared to that obtained in previous years. Inclusion of information concerning the routine activities of a histopathologic center were recorded along with data available from special investigations which were in progress during the year.

ROUTINE SPECIMENS RECEIVED: There were 9,926 specimens received in 1952. The type of material is listed below:

Human Autopsies	927
Surgical Specimens	8,477
Miscellaneous	522
	9,926

The work performed by the technical section during 1952 is detailed in the following:

Paraffin Blocks Prepared	15,991
Routine H and E Stains	34,592
Special Stains	4,937
Hematology Slides	353
	55,873

The total volume of material received in 1952 represented an increase of 1,342 specimens, or 15.9% over that received in 1951, with an increase of 116 autopsies (14.4%), 1,271 surgical specimens (17.6%), and a decrease of 49 (8.5%) miscellaneous specimens. The technical section experienced a reduction of 30,633 (36.1%) units of work in 1952 compared to 1951.

A large number of the specimens received represented material which was processed elsewhere and forwarded to the Department of Pathology for review. The decrease in technical work performed is a direct reflection of this. Accessioning, review, and forwarding of such material to the Armed Forces Institute of Pathology generally required an expenditure of time and effort on the part of officer and clerical personnel equal to that of cases processed in this laboratory. Protocols, slides, paraffin blocks and wet tissues of 1,060 autopsies and 4,146 surgical specimens were forwarded to the Armed Forces Institute of Pathology. This represents an increase of approximately 40% in the number of specimens forwarded during 1951.

Autopsies - Autopsy cases were received from Japan, Korea, Okinawa, and the Philippine Islands. Table I lists the installations performing these examinations. Approximately 50% (463) of these autopsies were performed in Korea. With few exceptions, these cases were processed at the First Medical Field Laboratory and forwarded to the Department of Pathology for review. Thirty-eight percent of the autopsies were performed in medical installations in Japan.

A significant increase in fatal non-battle injuries was observed in 1952. Accidental injury was numerically the second most frequent cause of death encountered in the autopsies during the reporting period. Suicide and homicide were observed much more frequently in 1952 than in the two preceding years. The proportionate increase in these cases was greater than the proportionate increase in cases of accidental

Table I. Organizations Performing Autopsies in 1952

AFFE (Former Japan Logistical Command).....	353
406th Medical General Laboratory	179
Osaka Army Hospital	37
141st General Hospital	20
382nd General Hospital	2
US Army Hospital, 8162 AU (Fukuoka)	42
US Army Hospital, 8164 AU (Kyoto)	9
US Army Hospital, 8165 AU (Sapporo)	15
US Army Hospital, 8166 AU (Sendai)	24
Johnson Air Force Base Hospital	3
Nagoya Air Force Base Hospital	4
Body Armor Team, 8204 AU	13
8th Hospital Group, 994	3
KOREA	463
1st Medical Field Laboratory	32
3rd and 14th Field Hospital (POW)	75
64th Field Hospital (POW)	130
11th Evacuation Hospital	47
21st Evacuation Hospital	19
22nd Evacuation Hospital	17
121st Evacuation Hospital	35
171st Evacuation Hospital	4
8055th Mobile Army Surgical Hospital	15
8063rd Mobile Army Surgical Hospital	12
8076th Mobile Army Surgical Hospital	7
8209th Mobile Army Surgical Hospital	26
8228th Mobile Army Surgical Hospital	40
1st Marine Medical Battalion	4
OTHER FEC	111
Rycom Army Hospital	80
Clark Air Force Base Hospital	29
Philippine Scout Hospital, 8148 AU	2
Total	927

deaths when compared with 1951 and 1950 (Table II). This increase in deaths ascribed to non-battle injury is believed to be associated with an increase in theater personnel strength. The cases of homicide include 25 POW's presumed beaten to death by their associates; no cases of this type were recognized in 1951.

As in 1951, infectious disease was the most frequently encountered cause of death due to disease during the year. This included 122 POW deaths and 51 cases of hemorrhagic fever* among UN personnel. Battle injury and disease caused approximately the same number of deaths in 1951 and in 1952.

Included among the undetermined causes of death was a large number of cases in which the final autopsy diagnosis was deferred until results of toxicological examinations were completed or reports of the circumstances surrounding death were received. Preliminary reports of investigation indicated that alkaloid poisoning was suspected in an appreciable number of these cases.

* Including cases with death in 1951, but received by this laboratory in 1952.

Table II. Summary of Causes of Death, 1950, 1951, and 1952

<u>Cause of Death</u>	<u>1950</u>	<u>1951</u>	<u>1952</u>
Non-Battle Injuries	219	264	345
Accidental	196	200	246
Suicidal	19	26	42
Homicidal	4	17	49
Circumstances Undetermined		21	8
Battle Injuries	115*	90	109
Disease	148	391	390
Infants	51	39	38
Undetermined	14	23	45

* Ten additional patients listed under "Disease" also had battle injuries

Table III. Causes of Death in 927 Autopsies in 1952

Non-Battle Injuries

Accidental	246
Trauma, Type not Stated	8
Train	3
Vehicular	53
Fall	6
Airplane	9
Gunshot Wound	33
Poisoning	46
Ethyl Alcohol	6
Ethyl Alcohol and Barbiturates	3
Ethyl and Methyl Alcohol	1
Ethyl Alcohol, Barbiturates, and Alkaloids	1
Alkaloids	14
Carbon Monoxide	10
Barbiturate	4
Halogenated Hydrocarbon	4
Ethylene glycol	2
Procaine	1
Electrocution	9
Anesthetic and Postoperative Death	10
Strangulation (machinery)	1
Parachute Fall	3
Drowning	38
Thermal Burns	16
Aspiration	5
Impaling	1
Explosion	4
Landslide	1
Suicidal	42
Gunshot Wound	27
Hanging	9
Carbon monoxide Poisoning	4
Fall	1
Stab Wound	1
Homicidal	49
Gunshot Wound	8
Hanging	3
Stab Wound	9
Trauma (includes 25 POWs beaten to death by cellmates) ..	29

Table III. Causes of Death in 927 Autopsies in 1952 Continued

Circumstances Undetermined	8
Hanging	3
Gunshot Wound	4
Trauma	1
Cause of Death Undetermined	45
Disease	
Cardiovascular and Renal	102
Arteriosclerotic Heart Disease (1) & (2)	46
Rheumatic Heart Disease	2
Rheumatic Heart Disease with Subacute Bacterial Endocarditis ..	1
Congenital Heart Disease	3
Myocarditis, acute and healed	3
Cardiac Decompensation (patent ductus arteriosus)	1
Aorta, syphilitic aneurysm	1
Aorta, dissecting aneurysm	2
Iliac Artery, Ruptured Aneurysm	1
Pulmonary Arteries, Thromboses	1
Polyarteritis Nodosa	2
Cerebral Hemorrhage (3) & (5)	31
Glomerulonephritis	5
Pyelonephritis	2
Nephritis, Type Undetermined	1
Alimentary and Biliary	29
Peptic Ulcer, Complications	3
Intestinal Obstruction	1
Perforated Viscus with Peritonitis	5
Intestine, Volvulus	1
Colitis, Chronic Ulcerative (amebic?)	1
Liver, Fatty Metamorphosis	2
Liver, toxic Hepatitis	1
Liver Abscesses and Pylephlebitis	1
Cholangitis with Liver Abscesses	2
Cirrhosis (4)	10
Pancreatitis, Hemorrhagic	1
Pancreas, Fibrocystic Disease	1
Infectious	222
Tuberculosis	40
Pneumonia, lung Abscess, etc.	28
Salmonella Infections	3
Bacillary Dysentery	1
Amebic Dysentery	2
Enterocolitis, etiology Undetermined	9
Tetanus	2
Relapsing Fever (?)	1
Meningitis, Purulent	5
Septicemia (6)	7
Hemorrhagic Fever	51
Infectious Hepatitis	15
Poliomyelitis	16
Japanese B Encephalitis	3
Encephalitis, Etiology Undetermined (7)	9
Paragonimiasis	5
Acute Bacterial Endocarditis	5
Aspergillosis	1
Necrotizing pharyngo-tracheo bronchitis	1
Gas Gangrene (9).....	1

Table III. Causes of Death in 927 Autopsies in 1952 Continued

Ascariasis	2
Brain Abscess	3
Mycotic esophago-gastritis.....	1
Infectious Disease, Etiology Undetermined	11
Neoplasm and Leukemia	22
Lymphoma	6
Carcinoma, Site (?)	1
Carcinoma, Lung	2
Carcinoma, Penis	1
Carcinoma, Liver	3
Carcinoma, Nasopharynx	1
Carcinoma, Stomach	1
Carcinoma, Rectum (8)	1
Neuroblastoma	1
Mediastinal Teratoma	1
Brain Tumor	4
Battle Injuries	109
Head	6
Neck	3
Thorax	11
Abdomen	13
Thorax and Abdomen	11
Extremities	19
Multiple	40
Burns	6
Infants	38
Stillbirth	6
Prematurity	14
Congenital Anomaly	2
Aspiration and Atelectasis	7
Tentorial Tear	3
Intestinal Obstruction	1
Diarrhea, Etiology (?)	2
Erythroblastosis Fetalis	2
Meconium Ileus with Peritonitis	1
Miscellaneous	15
Blood Dyscrasia	4
Malnutrition	8
Diabetic Acidosis	2
Asthma	1
(1) Incidental adenocarcinoma of rectum.	
(2) Rupture of heart with hemopericardial tamponade	
(3) One prisoner of war may have had a brain tumor, tissue lost.	
(4) One complicated by portal vein thrombosis.	
(5) Hemorrhage from either intracerebral hemangioma or from congenital aneurysm.	
(6) Kernicterus developed as a complication of sepsis in an infant.	
(7) 8 cases were unproven Japanese B encephalitis.	
(8) Case of congenital multiple polyposis with carcinomatous degeneration.	
(9) Inoculation wound.	

Surgical Specimens - A large number of the surgical specimens were of the routine type. Comparative totals of the more common types of specimens are listed for 1951 and 1952 in Table IV. Included in these totals for 1952 are the types and number of specimens received from Korean civilians, internees, and POW's. Tuberculous lymph nodes comprised a significant proportion of the total specimens received from this group of patients.

Table IV. Routine Surgical Specimens

	<u>UN Personnel</u>	<u>Koreans & POW's</u>	<u>Total 1952</u>	<u>Total 1951</u>
Appendices	920	8	928	1247
Endometrial Biopsies	513	3	516	404
Cervical Biopsies	531	4	535	385
Skin Biopsies	1601	83	1684	1623
Benign Nevi	529	5	534	348
Breast, Male	136	3	139	128
Breast, Female	124	-	124	143
Thyroid	65	1	66	76
Gall Bladder	91	11	102	78
Lymph Node	200*	128**	328	268
Pilonidal Sinus	101	-	101	61

* Tuberculosis - 4

** Tuberculosis - 62

There were 310 malignant tumors among the 8,477 surgical specimens received during 1952, an increase of 26.7% over 1951. Table V lists the diagnoses of the malignant tumors received during 1951 and 1952. Of the total, 47 were from Korean civilians or POW's. Gastrointestinal, squamous cell and metastatic carcinoma, and mesenchymal malignant tumors were the more frequent types of malignancy observed in this group.

Among United Nations personnel, an increase in the number of skin carcinomas, lymphomas and metastatic carcinomas was experienced during 1952.

Table V. Malignant Tumors Included in Surgical Specimens

	<u>UN Personnel</u>	<u>Koreans & POW's</u>	<u>Total 1952</u>	<u>Total 1951</u>
Skin & Mucous Membrane:				
Basal Cell Epithelioma	71	-	71	46
Squamous Cell Carcinoma	41	7	48	34
Precancerous (Bowen)	3	-	3	3
Malignant Melanoma	10	1	11	10
Breast				
Carcinoma	7	2	9	10
Gastrointestinal System				
Carcinoma, Esophagus	-	1	1	0
Carcinoma, Stomach	4	1	5	5
Carcinoma, Colon	5	2	7	3
Carcinoma, Rectum	5	3	8	0
Carcinoid	2	-	2	0
Primary Carcinoma, Liver	-	2	2	1

Table V. Malignant Tumors Included in Surgical Specimens Continued

	<u>UN Personnel</u>	<u>Koreans & POW's</u>	<u>Total 1952</u>	<u>Total 1951</u>
Genitourinary System				
Carcinoma, Bladder	6	-	6	4
Seminoma, Testis	4	-	4	5
Embryonal Carcinoma, Testis	6	-	6	5
Teratocarcinoma, Testis	1	-	1	5
Carcinoma, Cervix	12	2	14	9
Carcinoma, Uterus	2	1	3	3
Ovarian Tumor	1	1	2	4
Respiratory System				
Carcinoma, Larynx	1	-	1	1
Carcinoma, Bronchus	1	-	1	1
Bronchial Adenoma	1	-	1	0
Nervous System				
Brain Tumor	3	-	3	3
Paraganglioma	1	-	1	0
Lymphomas and Leukemias				
Hodgkins Disease	9	-	9	7
Lymphosarcoma	3	-	3	2
Reticulum Cell Sarcoma	2	2	4	3
Malignant Lymphoma, Unclassified	15	2	17	1
Leukemia, Granulocytic	1	-	1	0
Leukemia, Lymphocytic	1	-	1	5
Leukemia, Unclassified	2	-	2	3
Mesodermal				
Sarcoma	-	3	3	4
Fibrosarcoma	3	2	5	6
Dermatofibrosarcoma Protuberans	1	-	1	1
Osteogenic Sarcoma	-	1	1	1
Giant Cell Tumor	-	1	1	0
Other				
Carcinoma, Thyroid Gland	7	1	8	7
Mixed Tumor, Salivary Gland	16	1	17	16
Carcinoma, Salivary Gland	2	1	3	0
Ameloblastoma	1	1	2	0
Carcinoma, Sebaceous Gland	-	1	1	0
Adenocarcinoma, Palate	-	1	1	0
Metastatic Carcinoma	13	6	19	7
Undifferentiated Carcinoma	-	1	1	0
Total	263	47	310	227

Miscellaneous Specimens - Most of the miscellaneous specimens received (Table VI) were from a variety of experimental animals used in the attempted isolation of the causative agent of hemorrhagic fever. Also included were a number of tissues from laboratory animals dying from other causes in the experimental group. Included in this group were a number of animals with Tyzzer's or Theiler's disease. Although a number of animal brains were examined for the presence of Negri bodies, only four were positive (2 mouse, 1 dog, 1 cat).

Table VI. Miscellaneous Pathologic Specimens

Dog and Cat Brains Examined for Negri Bodies	54
Number positive	2
Mouse Brains for Confirmation of Rabies	17
Number positive	2
Guinea pigs for Tuberculosis	6
Experimental Animals	443
Total	520

BATTLE INJURIES: Specimens showing trauma from battle injury were received as surgical or autopsy material. There was a decrease in the number of surgical specimens examined during 1952, and a slight increase in the number of autopsy cases received. Table VII lists the types of specimens received during 1950, 1951, and 1952.

Table VII. Specimens Showing Trauma from Battle Injury

	<u>1950</u>	<u>1951</u>	<u>1952</u>
Surgical Specimens	208	249*	156
Autopsies	125	90**	109***

* Includes specimens from 9 POW's

** Includes 4 POW's who died of battle injuries

*** Includes 2 POW's who died of battle injuries

Table VIII lists the number of surgical specimens related to battle trauma received in 1950, 1951, and 1952. Although there were some variations in the type and volume of material showing injury in 1952, the significance of these variations could not be evaluated accurately. The factor of duplication of surgical specimens from patients who subsequently died and were autopsied was a minor variant because few such specimens were submitted for examination.

Table VIII. Types of Surgical Specimens Resulting From Battle Injury 1950, 1951 and 1952

<u>Site</u>	<u>1950</u>	<u>1951</u>	<u>1952</u>
Extremities	70	105	45
Battle Injury	37	60	43
Frostbite	33	45	2
Eyes	89	76	28
Central Nervous System	28	33	6
Lung and Pleura	-	12	8
Kidney	-	11	10
Spleen	9	7	19
Intestine	6	4	32
Arterio-Venous Fistula	6	2	8*

* Includes 1 POW

There was an overall decrease in the number of surgical specimens showing trauma. The number of amputated extremities in 1952 represented less than one-half the number

examined in 1951. The most significant reduction was in the number of extremities amputated because of frostbite. The two extremities examined were those of an Air Force crew member who suffered prolonged cold exposure after parachuting from his damaged aircraft.

A similar reduction was noted in the number of eye, central nervous system, lung, and pleura specimens examined. However, a slight increase in the number of traumatized abdominal viscera received was recorded. The eyes were not sectioned in this department, but in keeping with established policies were submitted to the Armed Forces Institute of Pathology for study.

AMPUTATED EXTREMITIES: During 1952, 66 amputated extremities were received by Pathology Department. Of these, 45 from 40 patients were the result of injury in battle. Forty-three specimens were from 29 wounded patients, and 2 specimens were from one patient with cold injury. The patient with cold injury later (in 1953) lost portions of both hands. The other 21 amputations were the consequence of non-battle injury. The incidence of amputation due to battle injury, especially multiple amputations, may show a significant increase in future action, because many men whose lives are saved by armored vests will have severe wounds of the extremities, some of which will require amputation.

Six cases were first examined elsewhere, and only the slides were forwarded for confirmation of findings. The other cases were consecutive cases amputated in Army hospitals in the Tokyo-Yokohama area. The distribution of the causes of amputation in this series had little statistical significance since most early amputations for destructive wounds were performed in Korea and were not submitted for pathologic examination. The group presented here includes a representative sample of the causes of amputation from three to forty days after injury.

Methods - After external examination of the extremity, certain sites were selected for detailed histologic and bacteriologic study. In general, when available, these were: (1) The original battle wound; (2) Muscle distal to the injury; (3) Muscle compartments proximal to, but not including, the wound tract. To obtain cultures away from the wound site, the overlying skin was cleaned with soap and water and incised with a sterile scalpel. The blade was resterilized by heating to incandescence, and specimens were obtained from the deep tissue. Companion blocks of tissue were taken, fixed in Zenker's solution, and stained with Hematoxylin-eosin for routine examination, and Goodpasture's stain for bacteria. The vessels and muscle groups were then dissected and described, and separate blocks for microscopic examination were taken as indicated.

Diagnosis - During the course of the year a variety of clinical and pathologic diagnoses were made on amputated extremities. These diagnoses showed little consistency. "Gangrene" was frequently used without indication of the cause. Review of these diagnoses permitted one to formulate a more consistent and useful set of diagnoses by stressing the major change which necessitated amputation. It was found that the recovery of pathogenic clostridia from a wound did not necessarily indicate clostridial infection, because the majority of wounds sustained in battle on the Korean front were contaminated with pathogenic clostridia. The sources of the contamination could easily be found in the soil and the soldier's clothing (1). In this study, culture reports were evaluated; (1) according to the portion of the wound which yielded the organisms, and (2) according to the distribution of organisms in the tissue as shown by the bacterial stains. Clostridia are large and were easily found. Unless organisms were numerous and located within tissue and not only in the exudate of a deep wound, their significance was discounted. All these cases suffered trauma, some degree of infection, and to a greater or lesser extent, some local impairment of the circulation. The diagnoses in Table IX indicate the dominant process in each case, i.e., the disorder which led to amputation.

The term anaerobic cellulitis was not used in this report, for no cases were found where clostridial infection was limited to the subcutaneous tissue. In the past some authors have used the term "anaerobic cellulitis (1)" to distinguish such cases from

gas gangrene which they called "anaerobic myositis". In this series, a distinction was made between two types of myositis, the spreading fulminating form (gas gangrene) and the localized form (localized clostridial infection).

Table IX. Final Diagnoses on Extremities Amputated as a Consequence of Battle Injury

<u>Diagnosis</u>	<u>No. of Extremities</u>
Gas Gangrene	3
Localized Clostridial Infection	2
Ischemic Gangrene, Uncomplicated	9
Ischemic Gangrene, with Secondary Mixed Wound Infection	6
Mixed Wound Infection Only	1
Extensive Trauma with Mixed Wound Infection	10
Trauma alone (Amputation for Mechanical Reasons)	11
Cold Injury	2
Unknown	1
	—
Total	45

Criteria for Diagnosis -

(1) Gas Gangrene - Although numerous objections to this term can be made, it has the advantage of wide usage. It signifies a wound infection by toxin producing, gas forming, anaerobic organisms of the genus Clostridia with profound systemic effects from the toxin, usually with hemolysis, rapid pulse, low blood pressure, pallor, fever (which is neither constant nor specific), and altered sensorium. The disease is progressive and may lead to death in 24 hours. Drainage from the wound is sero-sanguinous rather than purulent.

(2) Localized Clostridial Infection - In extremities classified as localized clostridial infection, the wounds contained large numbers of pathogenic clostridia growing in tissue and often producing gas. The local changes were like those of gas gangrene, but in contrast the systemic reaction was comparatively mild. Fever was present, but the prostration and toxicity of true gas gangrene was absent. The process was not progressive and amputation was not urgent, despite the alarming odor and gas from the wound.

(3) Ischemic Gangrene - This diagnosis was used when necrosis resulted from interruption of the blood supply. Frequently the devitalized tissue in these cases harbored various organisms, and occasionally purulent wound infection resulted.

(4) Mixed Wound Infection, Non-Gangrenous - This is the common type of wound infection and alone is rarely an indication for amputation. It is characterized by purulent exudate, and a septic course of variable severity. Mixed wound infection complicated many cases of ischemic gangrene and destructive wounds, and forced earlier and more extensive amputation than would otherwise have been necessary.

(5) Traumatic destruction of a mechanically indispensable portion of the extremity was a frequent indication for amputation. The extremities in this series listed as "traumatic destruction" were treated conservatively at first with the hope that part of the extremity could be saved.

Review of Characteristic Cases - 26808 - Gas Gangrene - This 23 year old American soldier was wounded by grenade fragments 13 October 1952, suffering a compound comminuted fracture of the upper third of the left tibia. When seen in a general hospital in Japan on 16 October 1952, his wounds had been debrided, but the

soft tissue of the calf was tense, necrotic, and contained bubbles of gas. His temperature was 103°, pulse 132, blood pressure 120/60. He was "very toxic", and appeared to be getting worse. Amputation above the knee was performed. The major vessels in the amputated portion of the extremity were patent and intact, and there was no history of femoral artery injury. Sections from deep in the gastrocnemius and soleus muscles showed necrosis, gas, and the presence of innumerable gram-positive rods without much neutrophil reaction. Cultures showed Cl. perfringens, Cl. novyi, and Cl. aerofetidum. The wound margin showed Cl. novyi, Cl. sporogenes, Cl. multifementans, beta hemolytic streptococcus, and a multitude of saprophytic aerobes.

26980 - Gas Gangrene - On 19 October 1952, this 22 year old American soldier sustained severe shell fragment wounds with bilateral traumatic amputation of both legs at the level of the knees and a compound comminuted fracture of the left forearm. He was wearing body armor, which very probably saved his life. His wounds were debrided a few hours later, and the stumps were placed in a plaster cast. He arrived at a general hospital in Japan on 23 October. At that time, he appeared "very toxic" and his temperature varied from 97° F to 100°F. The cast on the right leg stump was very tight, and the right thigh was swollen and crepitant. Despite systemic antibiotic therapy, irrigation with H₂O₂ and surgical exposure of the affected tissue, he became worse in 48 hours. A high femoral amputation was performed. The muscle was soft and dark reddish-grey. Sections showed necrosis and innumerable spore-forming gram-positive rods in the tissue. Cultures from four sites away from the exposed tissue bordering the wound showed Cl. sporogenes in pure culture. The wound border showed no Clostridia, but contained beta hemolytic streptococci. The H₂O₂ therapy probably eliminated the anaerobes bordering the wound.

This case clinically appeared to be severe gas gangrene, and the appearance of the tissue with swollen, edematous dark-red necrotic muscle, was also typical of the type of gas gangrene which shows little gas. The pure culture of Cl. sporogenes was of particular interest. Not generally considered to be a pathogen, the organism is markedly proteolytic and frequently is found associated with Cl. perfringens in typical gas gangrene. This case suggests that Cl. sporogenes alone may produce the clinical picture of gas gangrene, though the hemolytic systemic effect was notably absent.

27126 - Localized Clostridial Infection - This 21 year old American soldier sustained a compound comminuted fracture of the left forearm and a severed radial artery when struck by a shell fragment on 22 October. The wounds were debrided the next day. His course was uneventful, with a gradual rise in hemoglobin and general improvement. A mild febrile period lasted until about 30 October. The dorsum of the hand was black, swollen, and crepitant, and there was necrotic tissue near the wound margins. Amputation was performed through the fracture site 9 days after injury. The subcutaneous tissue and muscle of the hand showed edema, necrosis, gas bubbles, and numerous bacteria which on culture proved to be Cl. perfringens, Cl. sporogenes, and Cl. multifementans.

26708 - Ischemic Gangrene with Secondary Mixed Wound Infection - This 22 year old American soldier suffered shell fragment wounds of both legs on 1 October with a compound fracture of the lower one-third of the left tibia and a wound of the right popliteal space with a severed popliteal artery. The wounds were debrided. On arrival in Japan on 5 October, there was severe distension of the right calf. Fasciotomy was performed. For ten days he was treated conservatively, with no improvement or extension of the process. His temperature was moderately elevated with a daily spike to about 102°F. The extremity was amputated just below the knee on 15 October. It showed marked pallor of the large muscle. The distal half of the leg muscles and the subcutaneous tissue about the ankle and foot were edematous; and the overlying skin presented large fluid-filled blebs. Sections showed many gram-positive rods in the edematous tissue and cultures proved them to be Cl. sporogenes. Proteus morgani non-hemolytic streptococcus, and various other aerobic organisms were also recovered.

27422 - Severe Destructive Wound with Mixed Infection - On 1 November, this young Thailand soldier sustained a traumatic amputation of the right foot, and a massive compound comminuted fracture of the left femur and knee. He did not receive surgical care until 3 November, when his wounds were debrided. He arrived in Japan on 4 November. Despite 600,000 u/day of penicillin and 2.0 g/day of streptomycin, he ran a septic course with a daily spike of temperature to 104°F. The right leg stump was purulent and on 14 November, the extremity was re-amputated above the knee. The specimen showed purulent infection of the stump which extended upward several inches, forming a purulent necrotizing tract just posterior to the interosseus membrane. Cultures showed Cl. perfringens, Cl. novyi, and Cl. sporogenes, but tissue sections localized them in a narrow zone of necrotic tissue adjacent to the infected tract. Aerobic, alpha, beta, and gamma streptococci and anaerobic alpha hemolytic streptococci were also isolated.

Discussion - Most impressive in this study was the low morbidity and mortality from gas gangrene among wounded patients in Korea. Examination of amputated extremities yielded only three confirmed cases in 1952, and there were no autopsy confirmed deaths in Korea or Japan. The significance of this may be appreciated when the figures are compared with those of previous wars. The incidence of gas gangrene in the American Expeditionary Force in World War I was 4.76% of 187,936 wounded, and the mortality was 45 to 50%. MacLennan⁽¹⁾ reported an incidence of gas gangrene of 0.34% in 37,699 casualties in the North African campaign, and a mortality rate of 39% to 70%, depending upon the causative organism. The mortality rate in North Africa was essentially that of the first world war, but the incidence was much lower. Data for Europe and the Pacific in World War II were sketchy, but indicated an incidence of about 0.51% based upon published reports of 103,065 wounded (2,3,4,5). As shown in Table X, mortality figures were not reported in the larger series.

Table X. Incidence of Gas Gangrene in the European and Pacific Theater in World War II

Author	Wounded	Cases	Deaths	Gas Gangrene Incidence	Mortality
Odom	92,030	445	Not Reported	0.48	--
Power	6,000	20	2	0.33	10%
Neel and Cole	984	7	2	0.71	28%
Langley and Winkelstein (Their American Cases Only)	4,051	32	3	0.79	9.4%
Total	103,065	504	7		
Overall incidence of gas gangrene				0.5%	
Overall mortality of gas gangrene (only 59 cases)				12%	

These observations indicated that we should have expected from 3 to 5 cases of gas gangrene per thousand wounds, with a case mortality of from 10 to 50%. Surgical care in the allied forces in Europe and Italy was generally good. Despite this, the incidence of gas gangrene was about 5 per thousand wounded in those theaters. Prompt evacuation was practiced there just as it is in Korea. There were two techniques in use in Korea that were not utilized extensively in World War II. One of these was primary anastomosis of several arteries. There is little doubt that this procedure saved some limbs, but there remained many wounded who suffered severe injury to the extremity but did not have a major vessel damaged, or who suffered inoperable wounds of the major vessels. In these wounded, vascular surgery could play no part in the prevention of gangrene. The therapy which was new in Korea, and was common to all wounded, was the early administration of procaine penicillin G. Numerous in vitro (6) and in vivo (7) (animal) tests have indicated this drug's usefulness in the prophylaxis of gas gangrene. Clinical experience in Korea seems to substantiate these experimental observations.

Frost-Bite: During 1952 two specimens resulting from frost-bite were added to those previously received by this laboratory. The present report is a more detailed description of the microscopic alterations in examples of cold injury which occurred during the winter of 1950-1951. These histologic changes were not different from those described by others. In selected instances, elastic tissue and muscle stains were utilized.

As previously stated (1951 Annual Report) at the line of demarcation there was considerable variation in width, amount, and type of cellular reaction. No distinct relation of this positional variation to the duration of exposure, the interval between exposure and operation, the presence of battle injury, or the type and amount of bacteria could be determined. The inaccuracy of history and the scanty clinical information imposed a large variable which would not occur in controlled animal experiments.

Subcutaneous Tissue - Skeletal muscle and subcutaneous tissue manifested more apparent pathologic changes than nerves, tendons or vessels. The changes in arteries and veins are considered separately. The earliest alterations of the subcutaneous tissue were studied in a specimen removed approximately 36 hours after cold injury. The tela subcutanea was widened by protein rich exudate, small blood vessels were engorged, and scattered leukocytic reaction occurred. In specimens with greater duration between exposure and operation, the inflammatory reaction became intense and as the level of delineation was approached abscesses were formed. Contiguous nerves, muscles and tendons were rapidly engulfed and near the line of demarcation, these disintegrated into pools of necrotic debris. Though this intense inflammation and necrosis occurred in the subcutaneous tissue, the corium and epidermis were seldom as severely involved. Sloughing, acute non-specific dermatitis and bullae were present in the immediate vicinity of the zone of demarcation. Distinct variations in the width of this epithelial reaction were not unusual.

Alterations of fat were rapid in onset but progressed slowly. Atrophic changes characterized by the increase of small vacuoles in the granular amphophilic cytoplasm resembled serous atrophy. Large macrophages with plentiful granular cytoplasm were mixed with lymphocytes and plasma cells in the perivascular and septal connective tissue.

Distal to the line of demarcation, all structures had a bland eosinophilic "ghost" appearance and remained intact. All cellular detail was lost but elastic tissue lamellae were identifiable by means of selective stains. When mummification had been present for a week or more, only a narrow band of tissue distal to the level of demarcation presented the ghost outlines. Further distally, inspissation, collapse, and dehydration caused loss of all architectural outlines.

With greater lapses of time following frost-bite, varying degrees of fibrosis occurred. Proliferating fibrous tissue formed narrow collars about vessels, nerves, tendon sheaths, and dermal appendages. The inflammatory response was gradually displaced and eventually confined to the line of demarcation. The predominant cellular reaction remaining proximal to the area of fibrosis was mononuclear whereas granulocytes and granulation tissue predominated between the fibrous tissue and the level of demarcation. At about the sixth week following exposure, the epithelium proximal to the delimiting zone was hyperkeratotic, possessed deep slender rete pegs and presented atrophy of the prickle cell layer. The dermal papillae were edematous and contained hemosiderin granules both free and within phagocytes. The subcutaneous fat manifested serous atrophy and separation of the lobules by bands of fibrous tissue. This overall fibrosis encased all structures including the few atrophic dermal appendages.

Nerve - No special stains were employed to determine myelin or axis cylinder destruction. Large nerves in the anterior and posterior tibial vascular sheaths showed few changes. In an occasional example, there were isolated small clusters of large mononuclear cells probably myelophages. Small nerves in the neighborhood of the zone of demarcation manifested few alterations. Inflammatory reaction remained perivascular and usually localized in the perineurium. At the delimiting line, cellular infiltration increased, variations in staining quality occurred, and eventually eosinophilic "ghost" structures appeared. Beyond the zone of demarcation, the alterations resembled bland infarction.

As the duration of time between exposure and amputation increased, two changes became noticeable. Small vessels, chiefly arteries and arterioles, in the perineurium and within the nerve fiber were thickened by an increase of smudgy media. The other apparent modification was an increase of fibrous tissue in the perineurium and about small vessels, forming thin haloes infiltrated by small mononuclear cells. No specimens beyond six weeks duration were available for study.

Bone - Three sections of bone were available for study. In one instance, a metatarsal obtained three weeks after frost-bite, osteoid was absent, articular cartilage remained unchanged and the fatty marrow contained hemorrhages and was undergoing serous atrophy. The latter alteration was similar to that in the subcutaneous fat. At the end of five weeks, the findings were essentially the same. In one specimen of six weeks duration, the bone was acellular and osteoid was absent, becoming essentially a sequestrum.

Vessels - Arteries, arterioles, veins, and capillaries were examined in sections taken from all portions of amputated specimens. Arteries of large and medium size rarely presented more severe alterations than veins.

Veins equal to and larger than the dorsalis pedis seldom contained thrombi or manifested mesophlebitis except near the line of demarcation or near a suppurative wound. The thrombi were of several types. The most prevalent form was fibrinous and in varying stages of inspissation and organization. In a few instances, such thrombi were crescentic and incompletely filled the lumen. These examples were seldom associated with cellular reaction and suggested propagated thrombi. Blood clots in more advanced stages of organization plugged many venous tributaries and were capped by recent thrombi which extended into the larger veins. A less frequent thrombus was composed of tightly packed erythrocytes supported by a scanty network of fibrin. The veins containing such thrombi were invariably and markedly distended and were usually located near the zone demarcation. The most unusual occlusions were restricted to the immediate zone of delimitation and were composed of blood clots undergoing suppuration. Endophlebitis and mesophlebitis, especially the latter, were frequent and often associated with this form of thrombus. The inflammatory reaction in the media was composed of granulocytes and protein rich fluid and caused separation and distortion of the various medial components. In examples derived from the delineating zone, suppurative necrosis obliterated the muscular wall and in rare instances produced aneurysmal dilatations.

Large arteries (dorsalis pedis and greater) seldom manifested changes. Subintimal edema and isolated instances of endarteritis occurred. The latter was invariably composed of small mononuclear cells. Thrombi were usually crescentic, bland, and fibrinous, infrequently undergoing organization and rarely, puriform liquefaction. Smaller arteries near their origin were plugged by blood clots in varying stages of organization. From these thrombi, strands of a fresher nature entered the larger arteries (retrograde propagation). Mesarteritis was seldom present.

An unusual and striking alteration occurred in the inner half of the media in a few large arteries. Diffuse hyalinization with obliteration of cellular detail replaced this portion of the media and was not apparently related to mesarteritis, periarteritis or thrombosis. In these examples, subintimal spaces contained small and large mononuclear cells. The internal elastic lamellae in such specimens were frayed, duplicated and, in gaps, absent. These elastic tissue changes were more advanced than would be expected for the individual's age. In a solitary instance, an artery was markedly dilated and its media distorted and torn by pools of dissecting hemorrhage. Cellular reaction was sparse.

As the time interval between frost-bite and amputation increased, the pathologic alterations assumed a subacute and chronic nature. Thrombi occluding many of the arteries and veins were partially organized and a few vessels were recanalized. The muscular coats in these instances were often thickened by fibroblasts and proliferating capillaries. In specimens of longer duration, thin rings of proliferating fibroblasts encircled the vessels.

Smaller vessels, especially nearer the line of demarcation, presented more severe and more numerous pathologic alterations. In this zone, erythrocyte aggregates frequently distended the lumina and inflammatory reaction in the media was more intense. Small arteries and especially arterioles manifested thickened relatively hypocellular media. Whether this was edema, protein exudate or increased muscle density because of spasm or degeneration could not be determined. Those specimens which were more severely affected presented fraying, duplication, and gaps along the internal elastic lamella. In the few instances of fibrinoid degeneration of the media, the arteries were bathed by necrotic debris.

In cases of approximately six weeks duration, the vessels were thickened and more cellular than normal. Muscle bundles were separated and the interna elastica was seldom intact. A few vessels manifested capillarization of the media, but the prevalent finding was a fibrosing vasculitis, usually confined to the media and adventitia. Stains to differentiate fibrous from muscle tissue were not helpful in deciding whether some of the increase was of smooth muscle origin. In many instances, the alterations resembled thromboangiitis obliterans whereas other examples suggested encasement by fibrous tissue. Distal to the line of demarcation, the ghost structures of infarcts were seen and all vessels were dilated and filled with erythrocyte aggregates.

Muscle - Muscle was probably one of the most vulnerable tissues of the exposed limb. Alterations occurred at great distances from the line of demarcation and contrasted sharply in degree with adjacent vessels, nerves, and connective tissue septa. Granular and infrequently hyaline degeneration were the earliest histological changes identified. Cross striations were often well preserved in one part of the fiber while the other showed degeneration. Edema, congestion, and exudation followed shortly afterwards. As the severity increased, large patches of muscle manifested infarction and loss of cellular detail. These areas were encircled by narrow bands of cellular reaction at first granulocytic but later small and large mononuclear. The inflammatory response originated perivascularly and rapidly spread to the neighboring septa. In specimens of approximately 3 to 5 weeks duration, the cellular response was composed predominantly of large macrophages and proliferating granulation tissue. Uninfarcted muscle beyond this thin zone was atrophic as characterized by muscle giant cells, endomysial cell hyperplasia and loss of sarcoplasm. Two months after frost-bite, fibrous tissue had replaced infarcted muscle, had encased the few remaining atrophic muscle fibers, had greatly thickened the perimysium, and had formed dense adventitial collagen rings about vessels and nerves.

Tendon alterations were sparse; however, the tendon sheath was rapidly involved by immediately adjacent reaction in subcutaneous tissue. Near the line of demarcation, suppurative necrosis destroyed both tendon and sheath while distal to this zone, bland eosinophilic "ghost" structures were evident.

Fatal Battle Injuries - Of the 927 autopsy cases received in 1952 there were 109 from patients who died from battle injuries. As shown in Table VII, this represented a slight increase over the experience of 1951, and a considerable proportionate increase because the number of wounded in action and of men dying of wounds dropped sharply in 1952. As in 1951, the number of patients with battle injuries who died in Japan comprised only a small percentage (11%) of the total number of cases of fatal battle casualties. The battle fatalities included 12 soldiers who were killed in action and autopsied by members of the Body Armor Team. The remainder of the patients died in various medical installations in Korea. Obviously a large group of patients with battle injury expired in forward medical units, but were not autopsied because of lack of facilities and personnel for such examinations. However, available medical statistics indicate that approximately 24% of the patients who died from battle injuries in hospitals in Korea were autopsied.

The majority of the patients who died from battle injuries and were autopsied were members of the United States Armed Forces (97 of 109). Five were from other United Nations armed forces, and five were from the army of the Republic of Korea. Two were prisoners of war.

Information such as type of missile causing the injury, degree and duration of shock, interval between injury and resuscitation, and amount of blood given was not available in many cases and incomplete in others. With the exception of six burn cases, all of the injuries resulted from missiles, the majority of which were unqualifiedly described as shell fragments. The duration of life following injury in those soldiers who were not killed in action varied from one hour to 215 days. The patient who lived 215 days expired as a result of a cerebral injury. Table XII lists the immediate cause of death and the region injured in these cases. Frequently, injuries were multiple and severe and it was difficult to determine which was the lethal injury. In such cases, death was ascribed to multiple factors.

Death resulted from a number of factors at variable periods of time following injury. Circulatory collapse secondary to massive hemorrhage, extensive trauma, and injury to vital organs were the most common causes of death during the immediate hours following injury. In a few instances, deaths which occurred during surgery were ascribed to cardiac arrest during anesthesia. During the later post-operative periods, significant contributing factors in the cause of death were bronchopneumonia, intestinal obstruction, pulmonary embolism, sepsis, dehiscence of wounds, delayed hemorrhage, and acute renal failure (lower nephron nephrosis).

Table XII. Immediate Cause of Death and Region Injured

Cause of Death	Head	Neck	Thermal	Thorax	Abdomen	Thorax & Abdomen	Extremities	Abdomen & Extremities	Thorax, Abdomen & Extremities	Miscellaneous	Total
Brain damage	2										2
Brain abscess	3						1				4
Adhesive pericarditis				1							1
Operative cardiac arrest				1			1	2			4
Exsanguination-Shock		2	3	9	2	3	7	6	5	5	42
Exsanguination-Shock-Fat Emboli (?)							2				2
Delayed Hemorrhage & LNN						1					1
Rupture aorta (Concussion)						1		1			2
Carotid artery thrombosis		1									1
Pneumonia	1										1
Gastropleural fistula						1					1
Pulmonary embolus							2	1	1	1	5
Hemopneumothorax concussion								1			1
Peritonitis					1	2		1		2	6
Duodenal fistula					1						1
Intestinal obstruction					1			1			2
Peritonitis & LNN					3				1		4
Lower Nephron Nephrosis			1		5	2	4	2	3	4	21
LNN & Adrenal Infarct			1								1
LNN & Esophageal Perforation			1								1
Sepsis and LNN						1			1		2
Fat Emboli (?) & LNN							1				1
Sepsis-Fat Emboli (?) & LNN								1			1
Fat Emboli (?)								1			1
Sepsis							1				1
Total	6	3	6	11	13	11	19	17	11	12	109

Deaths occurring early in the post-injury period were clearly related to battle injury and circulatory collapse secondary to the trauma. Deaths occurring from complications later in the course of a patient's illness were not so clearly related and were ascribed to multiple causes. Common to this latter group were the problems of fat embolism and lower nephron nephrosis. The importance and relationship of these complications as related to battle injury are discussed in more detail in the following sections.

FAT EMBOLIZATION: Fat stains were done in a total of 80 cases of traumatic death for the purpose of identifying and quantitating fat emboli in the lungs, kidneys, and brain. The lungs were so studied in 75 instances, the kidneys in 65, and the brain in 42. Many, but not all, cases of death due to trauma were investigated using fat stains. Those cases were selected for special study which seemed by the type and extent of injury most likely to be associated with fat embolization or which showed suggestive evidence of fat embolization in sections stained with Hematoxylineosin. Therefore, any statistical conclusions as to the incidence of fat embolism in the various types of trauma observed can have only suggestive validity.

The number of fat emboli per section of lung, kidney, and cerebrum were graded one to eight-plus. One-plus was the grade assigned when careful search revealed only one to three emboli per section of organ; the eight-plus grade designated sections in which an estimated third or more of the capillaries and small arteries were filled with fat. Of the 49 battle trauma cases studied fat stains of lung sections were available in 44, of kidney sections in 41, and brain sections in 26. The entire series of 80 cases studied fell into four major groups as shown in Table XIII.

Table XIII. Types of Cases Studied with Fat Stains

	Number of Cases Studied with Fat Stains	Total Number of Cases
Wounded in Action	49	109
Vehicular Accidents	14	53
Deaths due to Beating	9	25
Burns	4	22
Miscellaneous	3	--

The 49 wounded-in-action cases were sub-divided according to the type, extent, and location of wound or wounds as follows:

Category 1.	One or very few missile wounds	6 cases
Category 2.	Multiple missile wounds (most commonly shell fragments)	43 cases
a.	Solely or chiefly involving extremities	13 cases
b.	Solely or chiefly involving trunk	8 cases
c.	Involving trunk and extremities	22 cases

In some cases in Category 2, the head and neck were also traumatized, but this was almost always minor compared to the injury to the trunk and extremities. In only one case was damage to the head an outstanding feature.

Of the six individuals with one or very few missile wounds, three showed wounds of the trunk alone, two of the trunk and extremities, and one of the head and neck alone. In none of these cases did there seem to be more than minimal soft tissue or osseous damage. The results of the fat stains shown in Table XIV suggested that in this category of trauma, fat embolization was not a prominent complication.

Analysis of the incidence and degree of fat embolization in Category 2 wounds suggested that trauma to the extremities much more frequently predisposed to significant fat embolization than trauma to the trunk. In the 12 cases of the former type

Table XIV. Incidence of Fat Embolization - Wounded in Action

Category	No. Cases Exam.	No. Pos. Cases	No. Cases Grade 5-8	Ave. Grade	No. Cases Exam.	No. Pos. Cases	Ave. Grade	No. Cases Exam.	No. Pos. Cases	Ave. Grade
1	5	4	0	2	5	0	0	2	0	0
2a	12	12	4	4	11	4	2	4	2	2
2b	6	5	0	3	7	2	0	6	0	0
2c	21	20	6	3	18	6	0	14	2	0

of injury there were 4 instances of moderate to marked (Grades 5 to 8) pulmonary fat embolization, while in 6 cases of the latter there were none. Likewise, of 11 cases of Category 2 in which the kidney was investigated by means of fat stains, 2 showed moderate to marked embolization, while in 7 cases of Category 2b none showed this degree.

As shown in Table XV, vehicular accidents were commonly associated with significant degrees of fat embolization. In these cases, again extremity injury seemed to be a frequent predecessor of the more severe grades of embolism.

Table XV. Incidence of Fat Embolization - Vehicular Accidents

	No. of Cases Examined	Number Positive	Number Grade 5-8	Average Grade
Lungs	14	14	7	4
Kidneys	9	6	0	1
Brain	6	1	0	1

Table XVI shows that deaths due to beating were accompanied by significant degrees of fat embolization more often than any other type of trauma in our autopsy material.

Table XVI. Incidence of Fat Embolization in Deaths Due to Beating

	No. of Cases Examined	Number Positive	Number Grade 5-8	Average Grade
Lungs	9	9	9	6
Kidneys	7	6	2	4
Brain	7	4	1	2

Although fat embolization has been reported in cases of burns, in our four cases where burns were the sole or chief form of injury, the degree of pulmonary fat embolization was minimal in 3 (Grade 1), and slight in the fourth (Grade #). The last case was also accompanied by laceration of the right thigh and avulsion of the right thumb.

The miscellaneous group of four cases includes some of unusual interest. One was of an individual who fell 28 feet from a balcony to gravel-covered ground below. He showed a Grade 5 pulmonary embolization. A second victim was in a bunker when he received shell fragment wounds to the left leg and foot; in addition, both legs were crushed when the bunker collapsed. His lungs showed a Grade 5 embolization. A third patient suffered a crushing type of injury of unknown etiology with incomplete amputation of both lower extremities; the lungs showed a Grade 7 embolization.

Table XVII shows the correlation of the incidence and degree of pulmonary fat embolization with the amount of osseous fracture incurred in the traumatic episode. The

Table XVII. Correlation of Incidence and Degree of Pulmonary Fat Embolization with Amount of Osseous Fracture

	Grade of Fracture			
	<u>0</u>	<u>1 - 2</u>	<u>3 - 4</u>	<u>5 - 8</u>
No. of Cases	20	21	17	12
No. Positive	17	20	17	11
No. Grade 5 or above	8	6	9	5
Average Grade	3.5	3.5	4	4

amount of fracture was graded 0 to 8-plus, using a point system. At one extreme, fracture of the pelvis or of the femur was given 10 points, while at the other a phalangeal, metacarpal, or metatarsal fracture was given a half points. Twenty or more points led to a grading of eight-plus; $\frac{1}{2}$ to 5 points, to a grading of one-plus.

It is obvious that there is no strict correlation between the degree of fat embolization and the amount of osseous fracture, if one disregards the additional presence or absence of soft tissue damage. Thus, the relatively high incidence of significant fat embolization in fractures Grades 0 - 2 is due to the inclusion therein of 6 cases of death due to beating, cases in which soft tissue damage undoubtedly played a major role. By and large, it was possible to correlate total soft tissue and osseous damage with the degree of pulmonary fat embolization. This correlation is not presented in tabular form because of the impossibility of grading accurately soft tissue damage from clinical and autopsy protocols.

As noted in the preceding tables (Tables XIV, XV, and XVI) renal and cerebral fat emboli were less commonly encountered than pulmonary fat emboli. As might be expected, the incidence of renal and cerebral involvement increases pari passu with increasing grades of pulmonary embolization. This relationship is shown in Table XVIII which correlates pulmonary and renal involvement in 64 cases in which fat stains of both organs were available.

Table XVIII. Incidence of Various Grades of Renal Fat Embolization Correlated with Graded Pulmonary Fat Embolization

		Grades of Pulmonary Embolization				
		<u>0</u>	<u>1 - 2</u>	<u>3 - 4</u>	<u>5 - 6</u>	<u>7 - 8</u>
Grades of Renal Embolization	0	2	14	19	2	0
	1 - 2	0	2	3	13	0
	3 - 4	0	0	0	3	1
	5 - 6	0	0	0	0	2
	7 - 8	0	0	0	0	0

A similar type of correlation of pulmonary and cerebral embolization was possible, although the latter as demonstrated in one or two sections of cerebral cortex was less common than renal embolization. In 42 cases in which kidney and cerebrum fat stains were available, the kidney was positive for emboli in 18, and the cerebrum in 9. In no case was the cerebrum positive when the kidney was negative.

Table XIX expresses the relationship between duration of life following trauma and the severity of fat embolization as observed at autopsy. Included in the group who lived one day or less was a number of DOA's, chiefly individuals beaten to death or involved in vehicular accidents.

Two factors may be implicated in the explanation of Table XIX. The obvious one is that injuries with extensive trauma to soft tissues and/or bone, the type commonly associated with high grades of fat embolization, were apt to be severe enough to cause

Table XIX. Relationship Between Duration of Life and Severity of Fat Embolization

Duration of Life to Nearest Day	Grade of Pulmonary Embolization				
	0	1 - 2	3 - 4	5 - 6	7 - 8
1 or less	1	4	6	14	5
2 - 3	0	2	5	3	1
4 - 5	0	1	4	5	0
6 - 10	0	1	5	1	0
11 or more	1	6	6	0	0

death in a relatively short period of time, while less severely injured patients might live for a number of days. Another possible explanation for the low incidence of moderate or severe fat embolization in those individuals who lived 6 days or more is that the intravascular fat may become metabolized or removed. The ultimate fate of fat emboli deserves further study. Individual cases in which the exact duration of life after trauma is known are of great interest in understanding the range of potentialities of fat embolization. At one extreme in this series was an individual involved in a vehicular accident, who lived for three hours and exhibited 5-plus pulmonary fat embolization, while at the other was a soldier who suffered shell fragment wounds of the extremities in addition to crushing trauma to his legs and lived six days. He, too, displayed 5-plus pulmonary fat embolization.

Perhaps the most important aspect of an analysis of fat embolization cases is the determination of its physiological importance to the patient and its part in the chain of events leading to death. All autopsy protocols and the available clinical records of 80 cases were reviewed in an attempt to determine whether respiratory, cardiovascular, renal, or neurological disturbances could be related to the degree of fat embolization in the lungs, kidneys, and brain. The cases of death due to beating which invariably showed moderate to severe pulmonary and varying degrees of renal and cerebral fat embolization, were accompanied by little or no clinical history. In only one case was an obvious cause of death (subarachnoid hemorrhage) discovered at autopsy. Although it is possible that the various physiopathological changes accompanying severe trauma may per se account for death, it is also conceivable that fat embolization is either the primary cause of death or plays an important role in such cases.

In the remaining cases, most of which had clinical histories, there were recorded no significant respiratory symptoms which could not be attributed to obvious and severe respiratory pathology unrelated to fat embolization. Likewise, at autopsy the lungs with moderate to severe fat embolization showed no consistent gross pathology and no consistent abnormalities in content of edema fluid or degree of renal fat embolization (Grade 7). He lived for two days and 15 hours following shrapnel wounds to the extremities; his urine output was said to be adequate during that period. This patient was also the only one who showed clinical evidence of cerebral damage clearly attributable to fat embolization. His clinical history was one of postoperative deep coma followed on the succeeding day by the onset of anisocoria, hypertension (blood pressure 170/100) and convulsions. Bilateral cranial trephining was done, but exploration was negative. At autopsy, the brain showed petechiae scattered throughout the cerebrum and midbrain and a 6-plus fat embolization microscopically. There was no other brain pathology.

In addition to the above case, there were two others in which clinico-pathological correlation suggested that fat embolization may possibly have played an important role in causing death. All three cases are presented in Table XX.

There was little in the way of positive evidence from this series that fat embolization played an important role in the course of the wounded patient's illness. On the other hand, in the wounded individual it was entirely possible that shock and the many other complications of trauma may have masked the manifestations of fat embolization, and that more refined methods of investigation are required to uncover them.

Table XX

<u>Type of Wound</u>	<u>Duration of Life (Days)</u>	<u>Mode of Death</u>	<u>Grade of Embolism</u>		
			<u>Lungs</u>	<u>Kidneys</u>	<u>Brain</u>
Shrapnel Wounds of Extremities	3	Postoperative coma	7	7	6
Vehicular, trauma to Pelvis and Extremities	1	Sudden death 12 hr. post- operative	6	3	4
Traumatic amputation of right leg, avulsive wounds left thigh, unknown etiology	?	Postoperative coma, cardiac arrest, follow- ing tracheotomy	8	5	Not Done

LOWER NEPHRON NEPHROSIS - Using the same diagnostic criteria as in 1950-1951, thirty-three (30.3%) of the 109 battle injury patients who died and were autopsied in 1952 had morphologic evidence of lower nephron nephrosis. In contrast, 36.6% of 215 battle casualties autopsied during the preceding 18 month period exhibited the histologic changes of lower nephron nephrosis. The incidence during 1952 is thus slightly lower than the mean for 1950-1951. It may be noteworthy that the renal changes were clinically regarded as a significant factor in mortality in a slightly greater proportion of cases in 1952.

	<u>1950</u>	<u>1951</u>	<u>1952</u>
Battle casualties autopsied	125	90	109
No. with histologic evidence of lower nephron nephrosis	43	35	33
No. with clinical evidence of lower nephron nephrosis	30	21	29
% with histologic evidence showing clinical signs also	69.8	60.0	87.9
% of total battle casualties with clin- ical evidence of lower nephron nephrosis	24.0	23.3	27.5

Comparison of the above figures suggests that a relatively constant rate of lower nephron nephrosis occurred among patients who died from battle injury and were autopsied. Obviously, the rates apply to only a small fraction of the total number of fatal battle injuries, since less than 25% of the patients dying in hospitals in Korea were autopsied. The data also suggest that clinicians were more aware of this complication of battle trauma in 1952.

Only three of the patients with battle injury complicated by acute renal failure died in Japan. The majority of the patients expired in Mobile Surgical Hospitals and Evacuation Hospitals in Korea, particularly at the renal failure center. At this latter hospital, facilities for hemodialysis were made available early in 1952 for the treatment of patients with acute renal failure associated with battle trauma or disease, e.g. hemorrhagic fever. The higher incidence of clinically recognized cases in 1952 was probably due in part to the stabilized type of warfare which permitted a more orderly evacuation and study of patients, and to the enlargement and improvement of clinical and laboratory facilities for the evaluation of these patients with acute renal failure.

Attempts to evaluate the relative importance of possible etiologic factors contributed little additional information. When the extensive use of blood in Korea and the large volumes of blood given to individual patients in many instances were considered, serious transfusion accidents with associated lower nephron nephrosis were found to be a rare occurrence. Although overt reactions were rare, the wounded in Korea

frequently received large volumes of bank blood with plasma hemoglobin levels greater than 40 mg/100 ml (See Chemistry Section this report). The significance of this infused hemoglobin in patients who developed acute renal failure has not been determined but apparently is not an important factor. Too few plasma hemoglobin levels were recorded on these patients who died to express any correlation between the clinical data and histologic observations available.

With few exceptions, all patients in Korea were given group "O" blood. The titer was marked "low" or "high" and those patients with blood groups other than "O" were given "low-titer" blood. Blood was given to all of the patients in this series. In many cases the amount given was not accurately known and in three cases was unknown. The average amount given to any one patient was 12 pints. The median was 10 pints with extremes of 2 and 40. These figures were slightly greater than the median of 7.0 pints recorded in 1950 and 1951 and probably reflected the greater availability of blood in many of the forward installations. In only one case was a transfusion reaction recorded and this patient received group "O" blood. His blood group was not known. A notation of a "slight transfusion reaction" was made in the clinical record. A period of hypotension followed this incident and bloody urine was observed. Since the patient had sustained a bladder wound which had been repaired, the significance of bloody urine could not accurately be evaluated. The degree of hemolysis could not be determined since plasma hemoglobin levels were not available on this patient. However, following this reaction, slight icterus was observed and death occurred 8 days later from lower nephron nephrosis and battle wounds. There was no evidence of trauma to the liver or biliary tract.

Most of these patients had severe battle injuries. Circulatory collapse resulting from the initial injury, subsequent trauma of surgery, dehiscence of abdominal wounds, or secondary hemorrhage was the primary indication for the use of blood in the majority of patients. From an etiologic standpoint, extensive wounds which required the use of whole blood, such as extensive soft tissue trauma, severe and often profound shock, and associated infections appeared more important in the production of acute renal failure than the factor of transfusion reactions in this group of patients.

Type and Extent of Injury: In this group of cases, there were 8 injuries caused by gunshot wounds (5 abdomen alone, 2 abdomen and thorax, and 1 the extremities alone). Twenty-four of the injuries resulted from shell fragment missiles, the majority of which were due to artillery and mortar shell bursts. In 3 patients shell fragment wounds resulted from the explosion of anti-personnel land mines. Also included was one case of renal failure which resulted from 2° and 3° phosphorus burns involving 30% of the skin surface area.

Tables XXI and XXII list the information available concerning the site and extent of trauma.

As in previous years, a good correlation was suggested between the presence of lower nephron nephrosis and the anatomical site injured. The commonest sites of injury which were associated with lower nephron nephrosis were the abdomen, extremities and thorax, in the order listed (Table XXI). In the group of miscellaneous or multiple injuries, these sites were almost uniformly involved. The thorax and abdomen were injured in 9 of the 11 miscellaneous cases; the extremities in 10 of the 11. Head, neck, and spine injuries, although observed once in combination with the above multiple injuries, were of relatively minor severity in comparison with the other injuries.

When the individual injured areas were tabulated by single regions this relationship was exaggerated (Table XXII). The commonest sites of injury in the majority of cases with lower nephron nephrosis were the abdomen and extremities, either singly or combined. These data probably indicate nothing more than the fact that the patients with these types of injuries sustained extensive soft tissue trauma, experienced severe and prolonged shock, required considerable blood and extensive surgery, and frequently developed infections. Most of these factors have been described as etiologic in the production of lower nephron nephrosis. The relative significance of

each could not be assessed, except that overt transfusion reactions were not important in this group of patients.

Table XXI. Lower Nephron Nephrosis and Anatomical Regions Injured

Region	1952				1950-1951			
	No. Cases	Cases With LNN	% of Region With LNN	% of LNN	No. Cases	Cases With LNN	% of Region With LNN	% of LNN
Head	6	0	0	0	56	3	5.3	4.0
Neck	3	0	0	0	2	0	0	0
Spine	0	0	0	0	8	1	1.25	1.3
Thorax	11	0	0	0	10	0	0	0
Abdomen	13	9	69.2	27.3	29	17	58.6	22.7
Thorax & Abdomen	11	3	27.2	9.1	20	9	45.0	12.0
Extremities	19	6	31.6	18.2	35	22	62.9	29.3
Abdomen & Fxtremities	17	4	23.5	12.1	18	9	50.0	12.0
Miscellaneous (Multiple)	23	11	43.4	33.3	32	14	43.7	18.7

Table XXII. Incidence of Lower Nephron Nephrosis in Relation to Individual Areas Involved*

Region	1952				1950-1951			
	No. Cases	Cases With LNN	% of Region With LNN	% of LNN	No. Cases	Cases With LNN	% of Region With LNN	% of LNN
Head	6	1	16.6	1.7	64	5	7.8	4.4
Neck	3	1	33.3	1.7	6	2	33.3	1.8
Spine	1	1	100	1.7	25	8	32.0	7.0
Thorax	42	11	26.1	19.0	47	16	34.0	14.0
Abdomen	61	24	39.3	41.4	81	42	51.9	37.0
Extremities	57	20	35.1	34.5	72	41	56.9	36.0

* Each area listed separately and patients with multiple wounds counted as a separate case for each area involved.

Table XXIII. Severity of Battle Injury - Lower Nephron Cases Only

	1952	1950-51
Severe	28	58
Moderate	5	20

The majority of injuries were classified as severe. Many of the patients no doubt would have died had no facilities for rapid evacuation, resuscitation, and surgical treatment been available. Many records did not include specific information regarding intervals between time of injury and resuscitation or between time of injury and surgery, but in 20 cases in which the approximate time intervals between injury and surgery could be determined, the average was 7 hours.

In 19 cases the shock state was described as existing from 1½ to 20 hours with an average slightly over 3 hours. Generally, the wounded were given plasma soon after injury and during transportation to the rear. Occasionally, blood was available as far

Table XXIV. Degree of Shock in Patients with Battle Injury -
Lower Nephron Cases Only

	<u>1952</u>	<u>1950-51</u>
Severe	22	36
Moderate	5	12
Not Recorded	6	30

forward as aid stations and the wounded were given blood as well as plasma. Evaluation of the degree of shock was not possible, since blood pressures were not recorded on many patients. Pulse rates, although frequently described as rapid, were not commented upon regarding quality in the majority of patients. The notation of severe or profound shock without other information was the commonest observation made.

Extent Of Infection Complicating Injury: Twenty-three of the 33 cases showed a significant degree of infection at the time of autopsy (Table XXV). In five cases the inflammatory process involved more than one area. Since the commonest sites of injury were the abdomen and extremities, it was expected that the most frequent combination was that in which a peritonitis was present in association with a cellulitis. In two cases, there was a coexisting empyema and peritonitis. Of the extremity injuries, vascular damage was of such magnitude in three cases that gangrene of the extremity below the area of injury resulted. There were no instances of gas gangrene in this group of patients. In both 1950 and 1951, gas gangrene was observed in 2 patients with lower nephron nephrosis.

Table XXV. Inflammatory Processes Present at Autopsy - Cases
with Lower Nephron Nephrosis Only

<u>Inflammatory Processes</u>	<u>1952</u>	<u>1950-51</u>
Peritonitis	15	42
Pleuritis	5	13
Cellulitis	10	20
Pericarditis	0	5
Brain Abscess	0	2
Meningitis	0	1
Pyelonephritis	4	0

There was an insufficient number of cases to state definitely that coexisting inflammation produced severer grades of azotemia and histologic evidence of lower nephron nephrosis. In addition, 14 of the 33 cases were dialyzed with the artificial kidney one or more times with lowering of non-protein nitrogen levels, so that this factor was inconstant. Although one-half of patients with infection demonstrated non-protein nitrogen levels over 100 mgm% and were classified as moderate or severe histologically (Table XXVI), a significant correlation with the group of patients without infection could not be made.

Four cases with severe azotemia and extensive infection were classified as having mild lower nephron nephrosis histologically. Three had peritonitis and one an abscess of the femoral canal. Three patients had been treated with the artificial kidney and the fourth died during dialysis. Deaths of these patients occurred on the 5th, 11th, 15th, and 30th days. The patient who lived for 30 days died from extensive peritonitis. Six days following his initial injury and subsequent oliguria, he had begun to diurese and continued with an adequate urinary output until death, excreting 850 to 3000 cc daily. Despite this urinary output, he had a rising non-protein nitrogen blood level which required two dialyses. Hyperkalemia was not experienced during this interval. Histological examination of the kidneys revealed few hemoglobin cases, little evidence of tubular necrosis and a moderate degree of acute pyelonephritis. These observations suggest that the peritonitis and pyelonephritis in this patient contributed

Table XXVI. Comparison of Severity of Azotemia and Morphologic Changes
In Cases with Infection and Without Infection

Morphologic Severity of LNN	Non-Protein Nitrogen Blood Levels (mg/100 ml)*					
	UNK	35-100	100-200	200-300	300-400	400-500
Infection:						
Mild	3	2	1		4	
Moderate	2		2	1	2	
Severe			1	2	3	1
No Infection:						
Mild	3		2			
Moderate				1		1
Severe	1		1			

* Highest recorded level attained

significantly to the recurrent azotemia, since the tubular epithelium was not appreciably altered histologically except in the focal areas of suppuration.

A common observation among those clinicians who were treating patients with acute renal failure with the artificial kidney was that blood non-protein nitrogen levels frequently rose more rapidly after dialysis than would be anticipated if no residual infection was present (8). A rapid rise of non-protein nitrogen levels after dialysis was therefore not infrequently interpreted as an indication that unrecognized infection existed which required surgical intervention and/or change in antibiotic therapy. These observations suggest that infection may assume a more significant role in determining the degree of azotemia in acute renal failure than is generally realized.

Clinical Course: All of the patients manifested some evidence of renal failure. Oliguria was the initial sign in practically all of the patients and became manifest by the first or second day after injury. Subsequently, significant degrees of azotemia were recorded in 27 of the 33 patients. In only 3 patients was evidence of hypertension recorded. In 2 patients uremic frost was observed. Twenty-nine of the 33 patients were diagnosed clinically, and of these, 18 were graded as having moderate or severe lower nephron nephrosis histologically (Table XXVIII). Of the 4 patients not diagnosed clinically, the histologic process was considered mild in three and moderate in one. Although clinical and laboratory evidence of acute renal failure was present in these 4 patients, apparently it was not considered a significant factor in the cause of death by the clinician.

Table XXVII. Clinical and Laboratory Evidence of Nephrosis

	1952 (33 Cases)	1950-51 (78 Cases)
Azotemia (history of oliguria not recorded)	1	2
Oliguria	6	10
Oliguria and azotemia	21	21
Oliguria and hypertension	0	1
Uremia with uremic frost	2	15
Oliguria, azotemia, and hypertension	3	3

The average duration of life was 8.4 and the median 7 days, with extremes of 3 to 30 days. Seventy percent of the patients died in 11 days or less. In 1950 and 1951, the median was 8 days and approximately 75% of the patients lived less than 10 days.

The duration of life in those patients who died and had mild degrees of histologic change in kidneys varied from 3.5 to 30 days with an average of 6 days. All had clinical evidence of renal failure, oliguria and azotemia. There was evidence to indicate that milder histologic changes were generally observed in the early stages

Table XVIII. Comparison of Severity of Histologic Change with Clinical Diagnosis

<u>Clinical Diagnosis</u>	<u>1952</u>	<u>1950-51</u>	<u>1952</u>	<u>1950-51</u>	<u>1952</u>	<u>1950-51</u>
Lower Nephron Nephrosis	7	16	10	11	10	0
Possible LNN	0	2	0	1	0	0
Uremia	1	5	0	0	1	1
Transfusion Reaction	0	1	0	1	0	0
Not Diagnosed	0	6	1	16	3	18

of the disease. Oliguria persisted until death in all patients except the one patient who lived for 30 days and subsequently died from his battle wounds and sepsis.

Fourteen of the 33 patients were dialyzed on the artificial kidney from 1 to 6 times with an average of 2.5 dialyses. These patients survived from 3 to 30 days following injury with an average of 12.1 days and a median of 11 days. These survival rates were slightly increased over those for patients not undergoing dialysis. Despite the considerable subjective and objective improvement resulting from hemodialysis in the majority of these patients with correction of the hyperkalemia and azotemia, the majority of patients died because of sepsis, massive wounds, and continued and prolonged "uremia" (8).

Pathology: The gross pathologic changes in the kidneys were similar to those described in patients dying from traumatic shock during World War II (9). No new observations were made. The weights of the kidneys were increased in the majority of the cases (Table XXIX.).

Table XXIX. Weight of Kidneys - Lower Nephron Nephrosis Cases Only

<u>Combined Kidney Weight</u>	<u>1952</u>	<u>1950-51</u>
200-300	1	6
301-400	4	16
401-500	10	27
501-600	9	18
601-700	2	1
725	0	1
Unknown	7	9

Of the 26 cases in which kidney weights were recorded, the kidneys varied in weight from 250 to 610 grams with a mean weight of 455 grams and a median of 450 grams. Nephrectomies were performed in 4 cases and in these patients the remaining kidney varied in weight from 350 to 450 gram. In 7 cases in which weights were unknown, the description "enlarged" was used in 5 cases. The average combined weights in 1950 and 1951 were 451 and 433 grams respectively. The median of 450 grams was the same for both 1950 and 1951.

The kidneys from patients living more than 10 days showed the greatest enlargement; kidneys from 11 of the 13 patients who lived for more than 10 days weighed over 400 grams. The largest kidneys, with combined weights of 600 and 610 grams, were from patients who lived 13 and 16 days respectively. Conversely, 6 of 13 patients who lived less than 10 days had kidneys weighing less than 400 grams.

Microscopically, the factors of tubular necrosis, interstitial inflammation, pigmented cases, and tubulo-venous thrombosis were studied in all cases and graded in severity from 1 plus to 3 plus. Necrosis of tubular epithelium was the most constant single feature. Hemoglobin cases were frequently quite numerous, and when considered in connection with tubular necrosis affected grading. Tubulo-venous thrombosis was observed infrequently, although evidence of phlebitis in areas of necrosis was not uncommon.

Attempts were made to determine the time of appearance of the interstitial inflammatory cell infiltrates and numbers of cases in relationship to progression of the lesion. The pigmented cases in five low-power fields (60X) were counted in the cortex and medulla of the kidney and correlated with the duration of the disease.

As shown in Table XXX, interstitial infiltrates were absent or minimal before the fifth day of the disease in the majority of cases. After the fifth day, the infiltrates consisting of lymphocytes, plasma cells, eosinophils, and infrequently polymorphonuclear leukocytes, became more numerous and extensive. They were located predominantly in the cortico-medullary junction, although in severe cases of longer duration they appeared about necrotic distal convoluted tubules in the cortex. In cases of more than two weeks duration, the infiltrates were fewer in number. They appeared in greatest numbers from the fifth to the fifteenth day of disease. The decrease in infiltrates after this time may be associated with resolution, or it may be that the patient who survived this long had less severe renal disease and would have recovered had it not been for their associated injuries and sepsis.

Table XXX. Time of Appearance of Interstitial Inflammatory Cell Infiltrates in Lower Nephron Nephrosis

Duration of Disease (Days)	Degree of Interstitial Inflammation			
	Absent to Minimal	Mild	Moderate	Severe
3-5	8	1	1	
6-10	3	2	2	1
11-15	1	2	2	3
16-19	1	2	1	1
30	1			

Pigmented cases were observed in large numbers before the fifth day and as late as the 19th day of the disease. There was no clear-cut evidence of significant decrease in the number of cases with progression of the lesion up to the 19th day. A correlation could not be made with onset of diuresis since diuresis occurred in only one patient in this series. No significant relationship could be expressed between the number of cases and the degree of azotemia. Generally, the collecting tubules contained larger numbers of pigmented casts than the upper portion of the nephron, although exceptions were not uncommonly observed. A clear-cut relationship did not exist between the duration of the disease and the number of cases in collecting tubules.

Table XXXI. Pigmented Casts and Duration of Disease

Duration of Disease (Days)	Cortex	Number of Pigmented Cases/5 Low Power Fields				
		0-25	25-50	50-100	100-150	150-200
3-5		1	3		1	
6-10		5	3	4		1
11-15		2	1	4	1	
16-20		1	2	1	1	
30		1				
	<u>Medulla</u>					
3-5		1		2	1	1
6-10			3	5	5	
11-15		2	1	2	2	1
16-20		1	2		2	
30		1				

Fat, periodic acid Schiff's reaction, Heidenhain, hemoglobin and iron stains were performed in 23 of the 33 cases. Using fat stains, Mallory demonstrated significant fat droplet alterations in renal tubular epithelium in patients dying 18 to 96 hours after injury and traumatic shock (9). In patients dying prior to and following this interval, these changes in tubular epithelium were significantly less. These changes were interpreted as the morphologic evidence of parenchymatous injury and functional impairment in patients with traumatic shock. Corresponding hematoxylin-eosin preparations did not demonstrate any distinctive alterations of tubular epithelium during this interval.

Fat droplets, measuring one to several micra, were commonly observed in ascending tubules, less frequently in distal convoluted and collecting tubules. An arbitrary classification of the degree of such changes in the cases with lower nephron nephrosis showed little variation from that observed in control sections from patients dying instantaneously or several hours after injury. However, only one of the patients with lower nephron nephrosis died in the 18 to 96 hour interval following injury, the period during which fat droplet alteration is presumably most intense.

Fat droplets were prominent in only three cases, and each of these patients lived less than 6 days after injury. Conversely, there were also three patients who lived less than 6 days who showed changes of minimal degree, less than that observed in control tissues. No correlation could be made between duration of disease or severity of morphologic changes and fat droplet alteration.

Control tissues from rats were used in conjunction with hemoglobin and iron stains on this material. Arthrocytosis was produced in rats by employing a method similar to that used by Rather, substituting human hemoglobin for rat hemoglobin (10). Within two days after injection of the hemoglobin, granules which stained with the same affinity as red cells with LaPehne and Dunn-Okajima stains, appeared in proximal tubular epithelium. Rats living for 48 to 72 hours also had demonstrable iron in proximal tubular epithelium. In none of the 23 cases of lower nephron nephrosis studied with these stains was there evidence of demonstrable iron or hemoglobin in tubular epithelium of the kidneys. The pigmented cases had some affinity for the hemoglobin stains in cases of short duration but the material was not unequivocally determined to be hemoglobin. This affinity of the casts for the stain became less prominent in the distal convoluted tubules and collecting tubules. In the collecting tubules, there was almost complete absence of affinity for the stains, the material staining gray to blue with the Dunn-Okajima stain in contrast to the bright orange erythrocytes. Periodic-acid Schiff's reaction and Heidenhain stains did not demonstrate any significant glomerular or tubular changes which were not observed with hematoxylin-eosin stains, except for evidence of basement membrane disruption in areas of tubular necrosis.

INFECTIOUS DISEASES: Infectious diseases were listed as the autopsy cause of death for 222 patients, including 95 prisoners of war.

Japanese "B" Encephalitis - Exact tabulation of JBE deaths is dependent on laboratory confirmation by serologic techniques. Such studies, however, were available in only three of the eleven cases of encephalitis which were categorized as consistent clinically and histologically with a diagnosis of JBE. These 3 patients were UN personnel. Of the unconfirmed JBE cases one death occurred in a UN soldier, while the remaining 7 cases were prisoners of war. The histopathologic changes were similar to those observed in patients who died during the epidemic of 1950 and were described in the Annual Report for that year.

Smallpox - In contrast with the 10 cases autopsied in 1951, there were none in 1952.

Salmonellosis - During 1952, 3 fatal cases of salmonellosis occurred among prisoners of war. The organisms isolated in 2 patients were S. enteritidis and S. paratyphi C. In the third case, no organism or definitive serological identification was made. The clinical course, pathological findings, and screening bacteriological examinations in the latter suggested S. paratyphi A infection.

This case was similar in all respects to the large series of salmonella fever patients reported in 1951. This 36 year old male was admitted to the hospital with a three-week history of cough, shortness of breath, headache, bilateral lower chest pain and watery diarrhea as frequent as eight times a day. The early stages of his illness were accompanied by sore throat and increased fatigue. There was no history of chills or fever. Physical examination revealed a temperature of 100°F, dehydration, emaciation, and moist rales in the left lung. He was treated with penicillin (dosage unknown). On the 5th hospital day, he developed bloody diarrhea and was treated with streptomycin, chloromycetin, and 500 cc. of whole blood. On the 8th hospital day, symptoms and signs of a perforated hollow viscus were noted and the following day laparotomy was performed. A perforation of the ileum 75 cm from the ileocecal valve was identified and 7.5 cm of small intestine was resected. The patient's course was stormy and he died nine days postoperatively.

The surgical specimen consisted of a 7.5 cm. segment of ileum containing a solitary 1 cm circular ulcer which had perforated. The ulcer was lined by a narrow band of granulation tissue heavily infiltrated by lymphocytes, granulocytes, and large mononuclear cells. A short distance from this zone, there was subcucosal edema and a light infiltrate of mononuclear cells. A lymph node from the mesentery revealed marked reticuloendothelial hyperplasia and acute perinodal inflammation.

At autopsy, the serosa of the entire ileum and the distal one-half of the jejunum presented a bluish discoloration. The first visible ulcerations of the mucosa were encountered 17.5 cms proximal to the site of anastomosis and extended to the ileocecal junction, becoming progressively more numerous and larger. The ulcers were discrete, had raised reddened borders, a gray base, and, in general, the greatest diameter was in the long axis of the bowel. In the terminal ileum, ulcers were of varying sizes, and marked inflammatory reaction with injection and edema thickened the entire intestinal wall. The largest ulcer encountered between the terminal ileum and the site of anastomosis was 2.5 cm in greatest diameter. There was some edema of the cecal wall, but no ulcerations were seen. Except for gaseous distention of the colon, there were no remarkable changes. Several ascarids were seen in both the large and small intestine. The microscopic alterations were similar to those in the surgical specimen but milder and more diffuse. The spleen weighed 425 grams and showed hyperplasia without focal necrosis. Lungs were congested and edematous. The liver showed non-specific changes.

The example of S. enteritidis septicemia occurred in December, 1951 but was not received by this laboratory until August, 1952. The patient, a 20 year old male, was admitted to the hospital with a one week history of chills, high fever, and headache. This was followed shortly by anorexia, diarrhea, chest pain, and a cough productive of white sputum. He vomited once four days prior to admission and had an episode of epistaxis the morning of his hospitalization. On examination, the physical findings included severe emaciation, marked icterus and some right upper abdominal tenderness. The white cell count was 4,800 with 59 percent polymorphonuclear cells and 37 percent lymphocytes. S. enteritidis was isolated from blood cultures. Peripheral blood smears showed no Borrelia. Temperature fell from 103.6°F to 97.2°F the day after admission and his clinical status became critical. Abdominal tenderness and diarrhea continued and he expired on the third hospital day. At autopsy, marked icterus was evident, a rash was absent. The spleen weighed 425 grams and showed marked hyperplasia of lymphoid follicles with scattered perifollicular areas of necrosis. The liver weighed 1800 grams and manifested congestion and moderate Kupffer cell hyperplasia. Lymph nodes were enlarged and the microscopic alterations were those of marked hyperplasia of reticulo-endothelial cells and filling of sinusoids by large free mononuclear cells and neutrophils. An acute leptomeningitis was also present. At autopsy, S. enteritidis was isolated in cultures of heart blood, bile, spleen, lung, and feces. Smears and silver stains of spleen failed to reveal B. recurrentis.

The third example of fatal salmonellosis was a patient with S. paratyphi C endocarditis and septicemia. This 27 year old prisoner was admitted to the hospital with a history of anorexia, generalized edema, and a daily afternoon fever. He was treated symptomatically for a month and released only to be readmitted about a month later.

At that time he complained of persistent anorexia, increasing dyspnea, non-productive cough, and diarrhea. On admission, his temperature was 100°F; his pulse was 104 and of "pistol-shot" nature. The heart was enlarged, and both diastolic and systolic murmurs were heard at the aortic area. Fluoroscopy revealed what was thought to be syphilitic aortitis with an aneurysm despite a negative serological test for syphilis. He was treated with digitalis, but there was no improvement. The possibility of rheumatic heart disease with sub-acute bacterial endocarditis was entertained; but multiple cultures until two days prior to his demise were sterile. Penicillin was empirically started a week before the patient's death. S. paratyphi C. was isolated from blood the day prior to his death. Chloromycetin was begun but the patient expired after the first dose.

At autopsy the heart weighed 575 grams and was dilated and hypertrophied. The leaflets of the mitral valve and the cusps of the aortic valve showed nodular fibrous thickening and fusion of the commissures. In addition, there were partially calcified vegetations on both valves and adjacent endocardium. Microscopically, these vegetations were composed of fibrous connective tissue and in some areas contained necrotic material in which were gram-negative amorphous masses resembling bacterial colonies. There was a depigmented infarct of the spleen and an organizing infarct of the left kidney.

Infectious Hepatitis - During 1952, a total of 162 liver biopsies were examined. Of these, 96 were needle biopsies submitted by the Hepatitis Center at the 8164th Army Hospital in Kyoto and the remainder, both needle and surgical excision biopsies, were received from a variety of the Far East installations.

A few of the Hepatitis Center biopsies were for diagnostic purposes. Thirty were done early and repeated later in the course of hepatitis in 15 patients to evaluate Vitamin B-12 therapy. The remainder of the biopsies were taken from individuals who had (1) evidence of persistent liver damage following hepatitis in the form of hepatomegaly, elevated serum bilirubin, or abnormal bromsulfalein retention, or (2) relapsing or recurrent disease.

Of the 30 Vitamin B-12 biopsies the initial 15 were taken from the 7th to 17th day of the disease, while the later ones were taken from the 34th to 42nd day. Microscopic examination disclosed no significant difference between treated and control individuals insofar as disappearance of evidence of cellular damage, resolution of exudate either in the portal spaces or within the lobules, or the degree of developing fatty metamorphosis was concerned.

Of the individuals with clinical evidence of persistent, recurrent, or relapsing hepatitis, the great majority showed microscopic evidence of persistent inflammation, usually with residual exudate in the portal spaces and focalization of exudate within the lobules. A minority showed an apparent or real increase in portal fibrous tissue. However, this was often limited to a portion of the biopsy and was not accompanied by alteration of lobular architecture. The diagnosis of cirrhosis was not justified in any case. Consistent correlation of specific changes in the liver with BSP excretion, elevated serum bilirubin, or clinically determined liver size was not possible.

An interesting histological sequel of hepatitis in the mid to late stages of the disease was the appearance of fatty metamorphosis in a certain number of cases. Though usually minimal, this was moderate in degree in some cases. Although it has been described earlier, moderate degrees of fatty metamorphosis were not seen in the present series until the 7th week (very few biopsies were taken from the 3rd through the 5th week). In one case lipid was so abundant that the Kupffer cells contained it in considerable amounts. Aside from its clinical significance, the fact that moderate fatty metamorphosis appears in some cases of hepatitis should be recognized by the pathologist, for in certain instances, especially those with accompanying portal fibrosis, the histological picture may easily be confused with early alcoholic cirrhosis.

Two other aspects of hepatitis which have been demanding attention of late are the hepatic pigment metabolism and the development of portal fibrosis and cirrhosis following hepatitis.

Pigment was seen constantly in the Kupffer cells and usually in histiocytes in the portal spaces in the early cases (7th to 17th day). The pigmented cells were at times aggregated into clusters and the pigment tended to be granular and golden-brown resembling hemofuscin, although some of the larger globules appeared to be bile. In the repeat biopsies, the pigment, when present, was often in clusters of cells. Although the golden-brown character sometimes remained, often the pigment had a dull greenish-gray pale hue and was amorphous.

In early biopsies an occasional finding was the spreading of the portal inflammatory exudate not only into the peripheral portion of the lobule isolating single or small groups of liver cells, but also from one portal space to another creating a picture simulating cirrhosis. In some of these cases, a number of the infiltrating cells were elongated, had the appearance of fibroblasts, and lay in an intercellular matrix which was homogeneous and hyaline. This material stained weakly or not at all with Masson's trichrome stain for collagen. In the repeat biopsies, this intercellular material was no longer seen. In a minority of them there appeared an apparent or real increase in portal fibrous tissue, which stained for collagen; in others the portal fibrous tissue was unremarkable suggesting that either the matrix laid down in the first few weeks of the disease had been absorbed or that liver cells had regenerated and filled the originally collapsed stroma. As stated above, no cases were seen in which the histological diagnosis of post-hepatitis cirrhosis was justified.

Thirteen needle biopsies were taken at the 64th Field Hospital from Orientals who were thought clinically to have Clonorchis Sinensis infestation and evidence of liver disease. In all but one of these the specific ova were stated to have been recovered in the stools. The histologic diagnosis on 6 of the 13 biopsies was "Liver tissue essentially unaltered"; in the remainder, there was only non-specific changes, such as mild inflammation or fibrosis of the portal areas. In no case was the clinical diagnosis confirmed by biopsy.

The remaining biopsies were done to establish diagnosis in a wide variety of clinical states. One, in a patient clinically diagnosed as having sarcoidosis, showed granulomas compatible histologically with sarcoid. In 2 other cases in which the clinical diagnosis of sarcoid was questioned, the liver tissue was essentially unaltered. In 2 cases the clinical diagnosis of infectious mononucleosis had been made. From one of these, a biopsy taken about 5 weeks after onset of the disease showed dense infiltrates of mononuclear cells in the portal spaces and occasional small foci within the lobules. This patient had abnormal liver function tests, but no hepatomegaly. In the other case, there was initial marked abnormality of liver function tests and spider angiomas; at the time of biopsy the liver function tests had returned to normal, but vague symptoms referable to liver disease and the angiomas remained. The biopsy showed small foci of necrosis within the lobules surrounded by mononuclear cell infiltrates.

Parasitic Infestations - As with tuberculosis, the overwhelming majority of cases were found in the prisoners of war, where the incidental autopsy discovery of parasites was exceedingly common. As noted in Table XXXII, parasitism infrequently was the actual or contributory cause of death. However, the not uncommon discovery of large masses of worms in an intestine without attendant symptomatology or apparent tissue reaction was quite astonishing.

Ascariasis - This parasite was by far the most commonly encountered at autopsy in the prisoners of war, but it was considered to be the major contributant to the cause of death in only two cases. In one fatal case, numerous ascari were found in the extra and intra hepatic bile ducts, pancreatic duct, and large bowel. A branch of the pulmonary artery likewise harbored an adult ascaris. The visceral sites of parasitic infestation were associated with secondary abscess formation (liver and lung) which were largely responsible for the patients' eventual demise. The other fatality showed a severe cholangiolitis and multiple hepatic abscesses produced by masses of worms clogging the biliary tree.

Table XXXII. Parasitic Infestations

	POW		UN	
	Cause of Death	Incidental	Cause of Death	Incidental
Ascariasis	2	80	0	6
Trichiuriasis	0	30	0	0
Paragonimiasis	5	2	0	0
Taenia saginata	0	2	0	0
Hookworm	0	7	0	0
Schistosomiasis	0	1	0	2
Clonorchis sinensis	0	2	0	0

Paragonimiasis - Five cases of fatal paragonimiasis were autopsied during the year, all in prisoners of war. Four of these had cerebral infestations in addition to the usual pulmonary involvement. Not infrequently, the initial symptoms reported by patients with secondary cerebral lesions were referable to the central nervous system, suggesting neoplasm or a primary brain abscess. One such case had been treated with dilantin for seizures and headache for several months following an illness onset characterized by chills and fever. Nine months after the first symptoms, he was hospitalized for abdominal pain and recurrence of seizures, shortly followed by a hemiplegia. At this time, a hard, tender mass was palpable in the abdomen. The autopsy disclosed extensive pulmonary and hepatic involvement with secondarily infected paragonimiasis lesions, which in the left lower lobe were productive of the epigastric "tumor" mass. There were numerous metastatic parasite lesions in the brain with cystification.

Trichiuriasis - The ova or adults of trichiuris trichiura infestation were found in the intestinal tracts of 30 prisoners of war. Without exception these were incidental observations at post-mortem without correlation to the case of death. Twenty-one of the cases likewise had associated ascariasis.

Sparganosis - During 1952, the diagnosis "Sparganosis" was made in 3 patients, all prisoners of war. Each patient was operated for a subcutaneous mass in the lower abdomen or groin; in one case the diagnosis was incarcerated hernia. When the mass was dissected, the surgeon in each case described a small tapeworm situated in a sac or burrow. The wall of the sac, when examined microscopically, showed only a narrow zone of fibrosis with a mild chronic inflammatory reaction. The worm itself showed little detail on chance cross section. In one case, the surgeon re-operated on a mass within skeletal muscle and found a similar condition. Scant history was provided in these cases, but in two cases the patient gave a history of eating raw snake meat. The only symptom reported in each case was the presence of a mass.

"Sparganosis" is the term applied to tissue infestation by the plerocercoid larva of certain cestodes of the genus Diphylllobothrium. The adult tapeworms are not found in man, but are frequently inhabitants of the intestinal tract of various carnivora. The ova passed in the stool hatch under favorable circumstances into actively motile, ciliated coracidia. Minute crustaceans of the genus Cyclops ("water fleas") ingest the coracidia, which elongate and acquire thick cuticles and prominent spines, the pre-cercoid larvae. When an infected Cyclops (the first intermediate host) is ingested by a susceptible vertebrate (the second intermediate host, commonly snakes, frogs, occasionally chickens and small mammals) (11), the larva migrates through the intestinal wall and body tissue of the new host, usually reaching subcutaneous tissue where it grows into the plerocercoid larva or sparganum. When the definitive carnivora host devours the second intermediate host, the sparganum grows into the adult Diphylllobothrium within the host's digestive tract, thus completing the cycle.

The plerocercoid larva or sparganum is a very hardy and adaptable organism, averaging 2 to 9 cm in length, and a few mm in diameter. If a vertebrate which is not a favorable host for the adult worm devours the sparganum, the parasite merely penetrates

the enteric tract and re-encysts, still as a sparganum waiting for a favorable definitive host.

In the Orient, sparganosis is usually caused by the plerocercoid larva of D. mansoni. Actually the species of sparganum cannot be determined except by feeding the worm to a parasite-free dog or cat. If the "take" is successful, species may be determined by examining the adult which develops in the artificially infected animal. There is good evidence that man may acquire the disease in one of three ways.

(1) He may ingest infected Cyclops inadvertently, in water from streams and ponds. In this case, man is a direct second intermediate host (11).

(2) He may ingest uncooked the flesh of one of the usual second intermediate hosts (12,13). The sparganum, finding itself in an inhospitable environment, may penetrate the soft tissue to re-encyst in the same form as it entered, or may encyst but suffer some degenerative changes and lose its scolex. This last form, formerly called Sparganum proliferum, is now generally considered to be a degenerating form of the common sparganum of D. mansoni (12).

(3) He may use the flesh of the second intermediate host as a poultice over an eye or an open wound. The versatile sparganum then migrates from the dead flesh to the new, living host. Faust observed experimentally the direct invasion of the conjunctiva of a dog by this route. (11)

Prisoners of War - As mentioned elsewhere, the most significant feature in the overall analysis of autopsies performed on prisoners of war and civilian internees was the relative high rate of deaths caused by infectious diseases. Of all deaths due to disease in the POWs, 63.5% were primarily due to infectious disease. The following abbreviated table indicates some general comparative aspects of infectious disease incidence, with a listing of the five leading entities.

Table XXXIII. Incidence of Infectious Disease Among Autopsied UN Personnel and POWs

	Number of Deaths UN	% of Infectious Disease Total UN	Number Of Deaths POW	% of Infectious Disease Total UN
Hemorrhagic Fever	51	40%	0	0%
Tuberculosis	5	4%	35	37%
Pneumonia, lung abscess, etc.	8	6%	20	21%
Polioomyelitis	16	12%	0	0%
Hepatitis	14	11%	1	1%
All Others (Infection)	33	26%	39	41%

It should be noted that these statistics are not critically significant. Autopsies were performed on the great majority of UN deaths due to disease, but only an approximation of complete post-mortem coverage was possible in the POW compound area. The high incidence of tuberculosis was perhaps the most striking feature of the POW autopsy analysis, with this being the causative mechanism of death in 37% of all infectious diseases, compared to 4% in the UN group. The POW tuberculosis mortality was actually higher, for there were a number of clinically evident cases which terminated in death but on whom autopsies were not performed. Tuberculous meningitis occurred in about one-third of all the POW tubercular deaths coming to autopsy.

RELAPSING FEVER: During 1952, a significant reduction in the incidence of relapsing fever in Korea was experienced (Table XXXIV) (14). All patients were POWs.

Table XXXIV. Incidence of Relapsing Fever in Korea

	<u>1951</u>	<u>1952</u>
January	2	2
February	0	1
March	30	0
April	11	0
May	24	2
June	55	1
July	25	1
August	12	0
September	4	1
October	5	1
November	6	0
December	1	-
	176	9

No proven fatal cases of relapsing fever were encountered during 1952. However, 3 additional cases were diagnosed among the 20 fatal cases of salmonellosis and cases with septic splenic infarcts received during 1951. Spirochetes were observed only in the areas of infarction with Warthin-Starry stains. All other viscera were negative for spirochetes. The pathologic changes in relapsing fever and its frequent association with salmonella septicemia were described in the annual report for 1951.

LIAISON WITH OTHER MEDICAL UNITS: As in previous years, good liaison was maintained with other medical units in the Far East Command. This was largely possible because most of the pathologists and laboratory officers became personally acquainted with members of the department during their temporary assignment to the 406th Medical General Laboratory for purposes of orientation.

CPC's were held at weekly intervals for members of the department and the local dispensary staff. Evaluation of cases at this conference determined selection for general distribution. CPC's were distributed weekly to 21 hospitals in Japan and Okinawa and to the 1st Medical Field Laboratory in Korea. In accordance with established policies, the 1st Medical Field Laboratory effected distribution to hospitals and medical installations in Korea. In September, the method of distribution of CPC's by routes was revised because cases were not always forwarded from one installation to another on schedule. To expedite distribution, one case was distributed weekly to all medical installations. Autopsy findings and clinical pathological interpretations were given in more detail in lieu of forwarding autopsy protocols and slides. CPC's were conducted at weekly intervals at two hospitals in the Tokyo area. Surgical pathology and tumor conferences were held at Tokyo Army Hospital also at weekly intervals.

During the year several consultant and visiting pathologists were in the theater. In April, Dr. R. Moore visited the department and gave lectures on testicular tumors, his impressions of hemorrhagic fever, and conducted a CPC at Tokyo Army Hospital. In September, Dr. Jesse R. Edwards, a consultant pathologist to the Surgeon General, gave a series of talks on various aspects of cardiac pathology during his tour of laboratory facilities in Japan and Korea. During October, Colonel Chapman Binford, USPHS, went to Korea to observe cases of hemorrhagic fever. Lectures on the "Pathology of Leprosy" were given by Colonel Binford for the members of the department and for medical officers of the 8228th MASH in Korea during his tour.

In December, a meeting was held with Japanese pathologists in Tokyo for the purpose of organizing a Society of Japanese and American pathologists. The inaugural seminar, the first of scheduled monthly meetings, was held on 27 January 1953, and was attended by 12 Japanese pathologists, 11 pathologists from the department, and 2 pathologists from Yokosuka Naval Hospital.

Histopathology sets were forwarded to 25 medical installations in Japan, Korea, the Philippines, and Formosa during the year.

OFFICER PERSONNEL: A total of 21 medical officers were assigned to the Pathology Department during 1952. As in previous years, the department served as a pool for medical officers with laboratory training. Following a period of training and orientation varying from two to four weeks, 12 of the officers were assigned to hospitals and laboratories in Japan and Korea. Four officers remained on continuous duty with the department throughout the year. One of two officers who returned to the Zone of the Interior was on continuous duty with the department for ten months. Two of the officers from the Zone of the Interior and one from the 1st Medical Field Laboratory, Korea, were permanently assigned to the department later in the year. Of these 21 officers, 3 who were on continuous duty with the department were certified board pathologists; one being certified during the year. Three of the officers arriving from the Zone of the Interior were certified board pathologists. Five of the officers were regular army and 16 were reserve officers. At the end of the year, there were 7 officers on duty in the department.

As in previous years, the turnover of medical officers was not considered a difficulty. The mutual advantages and cooperation resulting from personal contact and association with laboratory officers who were to be assigned to other medical installations in the theater were again demonstrated to be well worth the effort and time spent in orientation. A similar opinion has been expressed by personnel in other departments of the laboratory who frequently obtain information and material for their research and investigative activities from other medical installations in the theater. The cooperation of laboratory officers in the theater was excellent.

RECOMMENDATIONS: Administrative procedure effecting liaison between the pathologists and the investigating officer in the study of cases of death occurring outside hospitals has been outlined in Circular 5, GHQ, FEC, February, 1950. This circular directs the investigating officer to submit a copy of his report to the pathologist "in lieu of a clinical history". When this procedure is followed, a more accurate evaluation of the cause of death is usually possible and equitable decisions can be made in determining line of duty. It is recommended that such administrative procedure be included in army regulations.

MEDICAL ARTS SECTION

During 1952, the Medical Arts Section functioned as a part of the Pathology Department. The following work was accomplished by this Section during 1952:

Charts	1,100
Black and white negatives ..	3,260
Prints	17,194
Lantern slides	1,058

The above figures indicate an increase of 20% over that of 1951. The bulk of the work during the first two quarters consisted of photographs of burned patients in Tokyo Army Hospital, and routine photomicrography.

A photographer was placed on TDY at the 8063rd MASH with the Neurosurgical Team headed by Lt. Colonel A.M. Meirowski. Approximately 475 black and white, and color exposures were made of patients, before, during, and after surgery; copies were made of the x-rays of head wounds. This series also included photographs of sterile instrument trays and the arrangement of different types of dressing carts.

Upon completion of the Neurosurgical project, the photographer went to the 8228th MASH to take still photographs of the various sections of the Hemorrhagic Fever Center, and the surrounding terrain. During the first quarter of 1952, a large number of photomicrographs were made to illustrate the pathology of hemorrhagic fever. In June, a photographer was placed on temporary duty at the 8228th MASH to film, in color, motion

pictures of hemorrhagic fever patients in various stages of the disease to include gross autopsy specimens and laboratory procedures. He was also to obtain pictures of Korean terrain features and to document the trapping of field rodents for the collection of parasites. Two Signal Corps motion picture photographers were placed on temporary duty at the 8228th MASH in September to obtain additional motion picture footage. Approximately 4,000 feet of color film was exposed on this project.

The evacuation of patients from forward aid stations to rear area hospitals was photographed in May. This series showed the several types of helicopters used, loading and caring for patients and the special casualty carrying pods attached to the sides of the smaller helicopters.

In August, a photographic record was made of the work and equipment in the Department of Entomology in Tokyo, the Ecology Section in Omiya and the Taxonomy Section in Kyoto. Mosquito breeding areas were photographed in black and white and 35 mm color at Omiya along with pictures of the interior of the section and the personnel at work. A photographer was placed on TDY with the Taxonomy Section in Kyoto to photograph black fly breeding areas, and the Japanese artists at work with their drawings.

Color processing of both Ektachrome and Ansco Color films was started in October. This additional function, plus the new lighting equipment and a new dryer required more floor space. In December, an additional room and a store room were acquired.

DEPARTMENT OF MEDICAL ZOOLOGY

Current functions of the Department of Medical Zoology include routine identification of intestinal helminths and protozoa, gonadotropic hormone tests, diagnosis of malaria, identification of various parasitic specimens (including tissue forms) and certain zoologic specimens, examination of proctoscopic specimens for intestinal protozoa in cooperation with the Amebiasis Clinic of the Tokyo Army Hospital, supply of type specimens of parasite forms to Army medical laboratories in the Far East, and furnishing parasitic specimens to investigators outside the Far East.

SUMMARY OF SPECIMENS EXAMINED: The number of specimens handled and total examinations are indicated in Table I. Not uncommonly, more than one examination was made on some specimens. For example, several examinations were made on each proctoscopic specimen, and during part of the year two examinations were made on routine stools.

ROUTINE STOOL EXAMINATIONS: A total of 3,055 dispensary patients other than Japanese Nationals (chiefly Americans) were examined for intestinal parasites (Table II). Of this number 22.0% harbored one or more parasites, 10.2% had helminths, and 14.4% protozoa. The incidence of Ascaris lumbricoides, Trichuris trichiura, and hookworm were 5.6%, 2.9%, and 1.5%, respectively; (See Table II). The incidence of intestinal protozoa was relatively low: Endamoeba histolytica 2.7%, E. coli 6.4%, Endolimax nana 6.4%, and Giardia lamblia 2.3%.

A group of 926 Japanese dependents of Americans were examined, most of whom were applying for visas (Table II). Infected individuals were subsequently treated by dispensaries. The occurrence of specific helminths was as follows: Ascaris 26.1%, Trichuris 21.5%, Hookworm 4.9%, and Trichostrongylus sp. 15.6%. Other helminths occurred infrequently. The incidence of E. histolytica was only 3.9%, E. coli 12.3%, E. nana 11.9%, and G. lamblia 4.2%. As noted in previous years these Japanese Nationals did not harbor E. histolytica greatly in excess of the Americans, and, as is generally true, Strongyloides stercoralis was more frequently encountered in Americans. The infections of Trichostrongylus sp. were extremely light.

EXAMINATIONS FOR MALARIA AMONG UN TROOPS IN KOREA: A total of 2,032 blood smears from U.N. troops were examined. A large majority of these were positive, as only smears diagnosed as positive were normally referred to this laboratory. Only Plasmodium vivax was found.

Early in the year preparation of smears and diagnosis were noted to be inadequate, and a member of the department spent two weeks training a group of technicians of several Army laboratories in Hokkaido.

DIAGNOSTIC PROCEDURES FOR CLINICAL AMEBIASIS: Beginning in January and continuing throughout 1952, two members of the Department were in attendance at the Amebiasis Clinic of the Tokyo Army Hospital two mornings per week. The primary objective was to apply all possible procedures in establishing a diagnosis of amebiasis, including the microscopic examination of direct smears, permanently stained mounts of proctoscopic specimens, and concentrates prepared from normal stools by the formalin-ether technic. These findings combined with cultures for both amebae and bacillary pathogens will afford data for evaluation of procedures, and certain correlations of findings with clinical and pathologic observations.

SPECIAL IDENTIFICATIONS: A variety of zoologic as well as parasitologic specimens were received from various parts of the Far East Command. Included among these were fecal samples for identification or confirmation of helminth eggs and protozoan cysts; definitive parasites, either in toto or in histopathologic sections; pathologic specimens; snails from Korea; snakes from Korea; and other miscellaneous materials for identification (Table III).

MOLLUSCICIDE STUDIES - LABORATORY SCREENING TESTS: A total of 167 organic chemicals were screened for potential molluscicides. Of these, 97 were received from

Table I. Summary of Specimens and Examinations

<u>Type of Specimen</u>	<u>No. Specimens or Cases</u>	<u>No. of Examinations</u>
<u>ROUTINE EXAMINATIONS</u>		
Stool Specimens:		
American	4070	5758
Japanese (Dep. of Americans)	1047	1447
Japanese Nationals	575	575
Blood Smears for Malaria (Human)	1943	2032
Blood Smears for Protozoa (Bird)	400	400
Urines for Pregnancy	1255	1255
Urines for Neoplasms	10	10
Special Identifications	125	125
Amebiasis Clinic:		
Saline Smears	1126	1126
Hematoxylin Stain	631	631
Normal or Purged Stools	687	1277
TOTAL	11,869	14,636
<u>SPECIAL PROJECTS AND RESEARCH</u>		
Stool Specimens:		
For Epidemiologic Studies on Amebiasis	10,884	10,884
Parasitism for Americans in Far East	380	380
Seasonal Studies on Helminths	2,034	4,068
Skin Tests:		
For Schistosomiasis	2,500	2,500
For Ascariasis	346	346
For Amebiasis	33	33
TOTAL	16,177	18,211
Snails (<i>Oncomelania nosophora</i>):		
Collected Relative to Mollusca- cide Studies	120,000	120,000
Collected for Laboratory Re- search and Distribution	18,563	18,563
TOTAL	138,563	138,563

Koppers Corporation, 51 from Armour Laboratories, 16 from Monsanto Chemical Company, and 3 from Dow Chemical Company. Testing was done by means of the plate method (1), which involves exposing ten adult snails on filter paper in petri dishes to each of three chemical concentrations (1:1000, 1:5000, 1:10000). After 96 hours, the dead snails are determined by crushing, and the LD₅₀ is calculated (2). Among the 167 chemicals only six warranted further consideration (Table IV). All except one of these had an LD₅₀ of 1:20,000 or over, an arbitrary criterion for further testing under field conditions.

Four proven molluscacides were tested by immersing snails in dilutions ranging from 0.5 to 10 ppm. This evaluation was made because of its importance to certain

Table II. Summary of Routine Stool Examinations for 1952

	Americans		Jap. Nat. Dependents*	
	No.	%	No.	%
No. Examined	3055		926	
No. Parasitized	671	22.0	537	57.9
No. with Helminths	313	10.2	482	52.1
No. with Protozoa	440	14.4	233	25.2
<u>Helminths:</u>				
<u>A. lumbricoides</u>	170	5.6	242	26.1
<u>T. trichiura</u>	90	2.9	199	21.5
<u>Hookworm</u>	47	1.5	45	4.9
<u>Trichostrongylus sp.</u>	18	0.6	144	15.6
<u>E. vermicularis</u>	11	0.4	2	
<u>Taenia sp.</u>	2		0	
<u>H. nana</u>	3		0	
<u>C. sinensis</u>	3		3	
<u>S. stercoralis</u>	9		1	
<u>M. yokogawai</u>	1		4	
<u>Echinestome</u>	1		0	
<u>Protozoa:</u>				
<u>E. histolytica</u>	83	2.7	36	3.9
<u>E. coli</u>	194	6.4	114	12.3
<u>E. nana</u>	193	6.4	110	11.9
<u>I. butschlii</u>	12	.4	3	.3
<u>G. lamblia</u>	71	2.3	39	4.2
<u>C. mesnili</u>	4	0.1	1	0.1

* Japanese dependents of Americans

methods of applying molluscicides, such as impounded water and continuous flow applications. Of the four chemicals tested, DN-1 (dinitro-o-cyclohexyl phenol) was the most effective. The MLD with a 48 hour exposure was 2.5 ppm. (Table V). For sodium pentachlorophenate (Santobrite and Dowcide G) 10 ppm. were required for a 48 hour exposure, and 5 ppm. were required for Dowcide B (2, 4, 5 trichlorophenol, sodium salt).

MOLLUSCACIDE STUDIES - FIELD PLOT DILUTION TESTS: Of approximately 6,000 chemicals screen-tested on *Oncomelania nosophora* in the laboratory (3), possibly 50 warranted further screening under field conditions. During 1952, twenty-one chemicals were so evaluated. Some of these had been previously tested (3, 4) but in most instances the approximate MLD for field conditions was not adequately determined.

Methods - The procedure used in these tests is designated as a field-plot test, since graded amounts of the active ingredient of each chemical is applied to a series of ditch sectors, or plots. Long ditches possessing uniform surface terrain, dimensions, and a high snail population were selected. The weeds and grass were cut and removed from the sides and bottoms of the ditches which were then surveyed into 100 sq. ft. plots. Retainer dams were constructed between plots to prevent mixing of chemicals by rainfall. Pre- and post-treatment snail collections of about 200 snails were picked uniformly from each plot. As many chemicals as possible were applied in one day to assure a uniform exposure under the same environmental conditions. The chemicals were dispersed in water of such quantities as to afford a 200 ml. application per sq. ft. All evaluations were based on active ingredients when commercial formulations were used. Application was made by garden sprinklers.

Table III. Summary of Special Identifications

Type of Specimen	No.	Findings
<u>STOOL SPECIMENS:</u>		
Feces	41	(1) <u>Ascaris</u> , eggs; (2) Hookworm (2) <u>T. trichiura</u> ; (2) <u>S. stercoralis</u> (1) <u>M. yokagawai</u> (1) <u>C. sinensis</u> (3) <u>E. histolytica</u> ; (2) <u>E. coli</u> ; (3) <u>E. nana</u> (2) <u>G. lamblia</u> ; (2) <u>I. butschlii</u> (1) <u>Faciolopsis buski</u> eggs (1) <u>Trichomonas hominis</u> (2) <u>Ascaris</u> , adult worms
Egg-count for whipworm	1	Light infection
Peri-anal swab	3	No pinworm eggs found
Proctoscopic specimen	4	(1) <u>E. histolytica</u>
Feces (horse)	3	(3) <u>Ascaris equorum</u> eggs (3) <u>Trichostrongylus</u> sp. eggs
Feces (dog)	2	(2) <u>Toxocara canis</u> eggs
<u>TISSUE SPECIMENS AND FLUIDS:</u>		
Muscle	1	<u>Trichinella spiralis</u>
Sub-mental mass	1	Adult worm of <u>Wuchereria</u> (probably <u>W. malayi</u>)
Small intestine	1	<u>S. japonicum</u> eggs in pseudotubercles in submucosa
Intestine	1	No ameba found
Rectal biopsy	1	No ameba found
Dermal cyst	1	<u>Cysticercus cellulosae</u>
Chest fluid	1	No scolices of <u>Echinococcus</u> found
Sternal marrow	1	Negative for leishmaniasis
Gall bladder contents	1	<u>Ascaris</u> eggs found
<u>SNAILS:</u>	2	(2) <u>Melania</u> sp.
Snails (Korea)	4	<u>Viviparus malleatus</u> ; <u>Thiara</u> sp.; <u>Lymnaea auricularia</u> ; and <u>Acusta sieboldiana</u>
Snails (Korea)	17	(1) <u>Semisulcospira gottschei</u> (Martens) (1) <u>Semisulcospira multiscutata</u> (Martens) (1) <u>Semisulcospira nodiperda</u> (Martens) (1) <u>Semisulcospira coreana</u> (Martens) (1) <u>Semisulcospira libertina</u> (Gould) (1) <u>Littorina brevicula</u> (Philippi) (1) <u>Batillaria cumingi</u> (Crosae) (1) <u>Gyraulus albus</u> (Mueller) (2) <u>Lymnaea auricularia coreana</u> (3) <u>Acusta sieboldiana</u> (Pfeiffer) (4) <u>Lymnaea olluba</u> (Gould)
<u>SNAKES (From Korea):</u>	3	(3) <u>Agkistrodon halys</u> (mildly venomous)
<u>MISCELLANEOUS SPECIMENS:</u>		
Blood smear	3	(1) <u>Microfilaria</u> of <u>W. malayi</u>
Nematodes	3	Non-parasitic soil nematodes (adults)
Urine	2	(1) No amebae observed (1) <u>Colpoda</u> (ciliate) found.
Vaginal swabs	2	No parasites found
Fresh Water	16	(1) <u>Ciliata</u> (free living)
Others	11	
Total	126	

Table IV. Potential Molluscacides Determined by Plate-Tests

Company Serial	Chemical Name	Physical State and Solubilities	LD ₅₀
K1514	Di-(3-methyl-6-isopropyl phenyl) dithiophosphoric acid	Liquid - soluble in ether and acetone	1:33,000
K1834	Di-(o-m-p-isopropylphenyl) dithiophosphoric acid	Liquid - soluble in ether and acetone; slightly so in water	1:19,850
K1510	Di-(o-tert-amylphenyl) dithiophosphoric acid	Liquid - sudsy in water; soluble in ether and acetone	1:33,000
K1516	Di-(o-sec-amylphenyl) dithiophosphoric acid	Liquid - soluble in ether and acetone	1:24,000
K1633	Dichlorohexyl thiuram monosulfide	Solid - soluble in ether and acetone	1:15,000
CP2323	*Tetraisopropyl monothiono pyrophosphate	Liquid - soluble in ace- tone; emulsifys in water	1:71,000

* Causes an abnormal distention of snail from its shell

Table V. Relative Effectiveness of Molluscacides by Immersion Testing

Company Name	Chemical Formula	MLD*
DN-1	Dinitro-o-cyclohexylphenol	2.5 ppm.
Dowcide B	2,4,5 - trichlorophenol, (sodium salt)	5.0 ppm.
Santobrite	Sodium pentachlorophenate 79%; other chloro- phenates 11%	10.0 ppm.
Dowcide G	Sodium pentachlorophenate 75%; other chloro- phenates 13%	10.0 ppm.

* With 48 hours exposure

Results and Discussion - Data from the field-plot dilution tests during the spring are tabulated and graphically illustrated in Table VI and Figure 1; those of the fall trials are illustrated in Table VII. Theoretical curves appear on the graph for Santobrite and DN-1 only; adequate data were not available on the other chemicals.

During the spring tests there was a minimum of rainfall, whereas in the fall the first series of tests (including Santobrite, DN-1, Dowcide-B, Dowcide-G, and DN-2) were exposed to a 3-inch precipitation within four days after application. The effect of rainfall on molluscacidal effectiveness has not been fully determined. McMullen (5) states that heavy rains are undesirable. Our fall and spring data cannot be utilized to settle this matter since the onset of hibernation confuses the situation. No exact criteria are available for evaluation of this factor. There is evidence that toxicity is less quickly manifested in the fall (5, 6).

Table VI. Results of Spring (1952) Field-Plot Dilution Tests

Chemicals*	Pre-Treatment Samples (Controls)		Dosage** Gms/sq ft	Post-treatment Samples			
	% Dead	No. Snails		4 Days		14 Days	
				% Dead	No. Snails	% Dead	No. Snails
Santobrite	2.0	(244)	1.0 gm	79.5	(210)	99.0	(533)
	0.9	(214)	.8 gm	92.2	(337)	95.2	(488)
	2.8	(142)	.6 gm	15.9	(176)	100.0	(215)
	8.2	(182)	.4 gm	38.3	(167)	77.8	(217)
	3.6	(192)	.4 gm	39.6	(199)	85.5	(138)
	2.1	(290)	.2 gm	28.7	(344)	92.6	(190)
	2.5	(199)	.2 gm	27.5	(261)	92.6	(299)
DN-1	0.9	(206)	.1 gm	89.3	(254)	95.2	(340)
	2.5	(322)	.1 gm	83.4	(272)	94.4	(216)
	2.4	(209)	.2 gm	66.8	(226)	98.7	(320)
	2.1	(326)	.2 gm	95.4	(284)	96.4	(282)
	2.8	(107)	.05 gm	87.0	(216)	96.2	(347)
	1.4	(137)	.025 gm	97.7	(453)	89.5	(306)
	0.0	(158)	.0125 gm	05.0	(276)	27.0	(631)
Dowcide B	2.6	(76)	.8 gm	97.2	(219)	100.0	(317)
	5.2	(473)	.6 gm	97.0	(304)	100.0	(337)
	5.6	(142)	.4 gm	90.7	(130)	98.7	(158)
	2.0	(145)	.2 gm	97.4	(159)	96.5	(230)
	3.2	(468)	.2 gm	74.2	(298)	87.1	(420)
Dowcide G	7.0	(170)	.8 gm	91.3	(220)	99.3	(156)
	6.7	(238)	.6 gm	92.4	(397)	98.8	(85)
	5.1	(175)	.4 gm	83.1	(160)	100.0	(199)
	25.4	(173)	.2 gm	77.6	(130)	100.0	(327)
	3.3	(209)	.1 gm	94.6	(373)	97.0	(305)
2, 4, 5 Trichlorophenol	3.5	(505)	.5 gm	97.2	(287)	99.1	(703)
	6.3	(512)	.4 gm	96.5	(380)	99.5	(664)
	8.0	(512)	.2 gm	94.0	(135)	98.4	(700)
	21.9	(347)	.1 gm	95.2	(189)	94.9	(632)
Dinitro-o-sec butylphenol	10.5	(266)	.3 gm	92.6	(150)	97.8	(186)
	12.1	(222)	.2 gm	91.4	(210)	98.5	(408)
	12.7	(181)	.1 gm	92.3	(210)	97.7	(271)
	10.3	(310)	.05 gm	92.9	(170)	97.3	(149)
Para tert butylphenol	18.5	(496)	.6 gm	48.9	(249)	62.9	(486)
	10.8	(590)	.4 gm	34.8	(192)	57.1	(507)
	6.6	(698)	.2 gm	35.7	(235)	47.1	(715)
Dowcide 2-S	1.0	(200)	.1 gm	94.1	(1178)		
Calcium cyanamide	0.9	(219)	1.3 gm	48.4	(378)	79.2	(496)
CP 5	4.2	(216)	.8 gm	98.8	(254)	99.1	(232)
	1.9	(224)	.4 gm	76.8	(267)	66.8	(223)
	1.5	(209)	.2 gm	75.7	(239)	46.9	(211)
CP 536	3.5	(287)	.8 gm	97.3	(270)	99.1	(228)
	1.3	(236)	.4 gm	97.0	(204)	97.0	(235)
	5.1	(197)	.2 gm	95.4	(243)	83.8	(217)

Table VI. Results of Spring (1952) Field-Plot Dilution Tests Continued

Chemicals	Pre-Treatment Samples (Controls)		Gms/sq ft	Post-treatment Samples			
	% Dead	No. Snails		4 Days		14 Days	
				% Dead	No. Snails	% Dead	No. Snails
CP 849	1.3	(163)	.8 gm	16.4	(225)	7.7	(206)
	4.9	(205)	.4 gm	10.4	(250)	8.7	(206)
	2.5	(410)	.2 gm	10.5	(237)	11.5	(208)
CP 850	8.5	(272)	.8 gm	22.4	(303)	12.3	(210)
	4.7	(234)	.4 gm	14.5	(275)	7.6	(223)
	2.3	(184)	.2 gm	9.4	(211)	6.4	(170)
CP 4646	35.9	(92)	.8 gm	44.8	(225)	32.4	(225)
	3.3	(123)	.4 gm	29.8	(426)	13.9	(136)
	11.1	(280)	.2 gm	8.1	(123)	8.9	(191)
CP 1558	32.7	(297)	.8 gm	72.2	(256)	94.6	(263)
	14.1	(263)	.4 gm	68.0	(260)	93.3	(242)
	27.2	(140)	.2 gm	35.2	(366)	89.2	(241)
CP 2292	26.6	(271)	.8 gm	94.7	(343)	96.9	(265)
	47.5	(251)	.4 gm	81.1	(307)	87.7	(229)
	20.4	(603)	.2 gm	48.6	(374)	87.9	(281)
CP 2293	18.7	(386)	.8 gm	81.9	(516)	93.0	(245)
	22.9	(271)	.4 gm	66.9	(427)	79.7	(292)
	17.4	(167)	.2 gm	55.3	(338)	44.2	(242)
CP 2294	24.4	(246)	.8 gm	70.3	(351)	93.4	(215)
	25.4	(331)	.4 gm	65.7	(342)	76.5	(188)
	19.3	(203)	.2 gm	40.8	(475)	38.5	(296)
CP 2367	1.0	(106)	.8 gm	63.3	(322)	82.6	(248)
	0.0	(122)	.4 gm	60.1	(402)	47.8	(259)
	16.0	(207)	.2 gm	38.0	(200)	4.1	(286)
CP 3438	0.0	(159)	.8 gm	50.9	(495)	51.2	(238)
	1.0	(103)	.4 gm	42.0	(506)	39.5	(253)
	0.0	(87)	.2 gm	27.9	(455)	21.9	(237)
50% Ethyl Alcohol; 50% EM 65***	7.8	(491)	.2 ml	1.9	(205)	2.6	(228)
Skil 234B***	8.4	(484)	1.0 ml	29.8	(482)	57.2	(473)

* The several entries under each chemical represent individual plot tests

** Active ingredient

*** Detergents used in dispersing molluscicides.

Temperature does not appear to be a critical factor in these two surveys since the average temperatures for the spring and the first fall period of application were about the same, with the fall temperatures tending to be a little higher. It is of interest that Santobrite gave near-maximum kills as soon as four days after application under the influence of heavy rains, whereas in the spring under dry conditions

GRAMS PER SQUARE FOOT

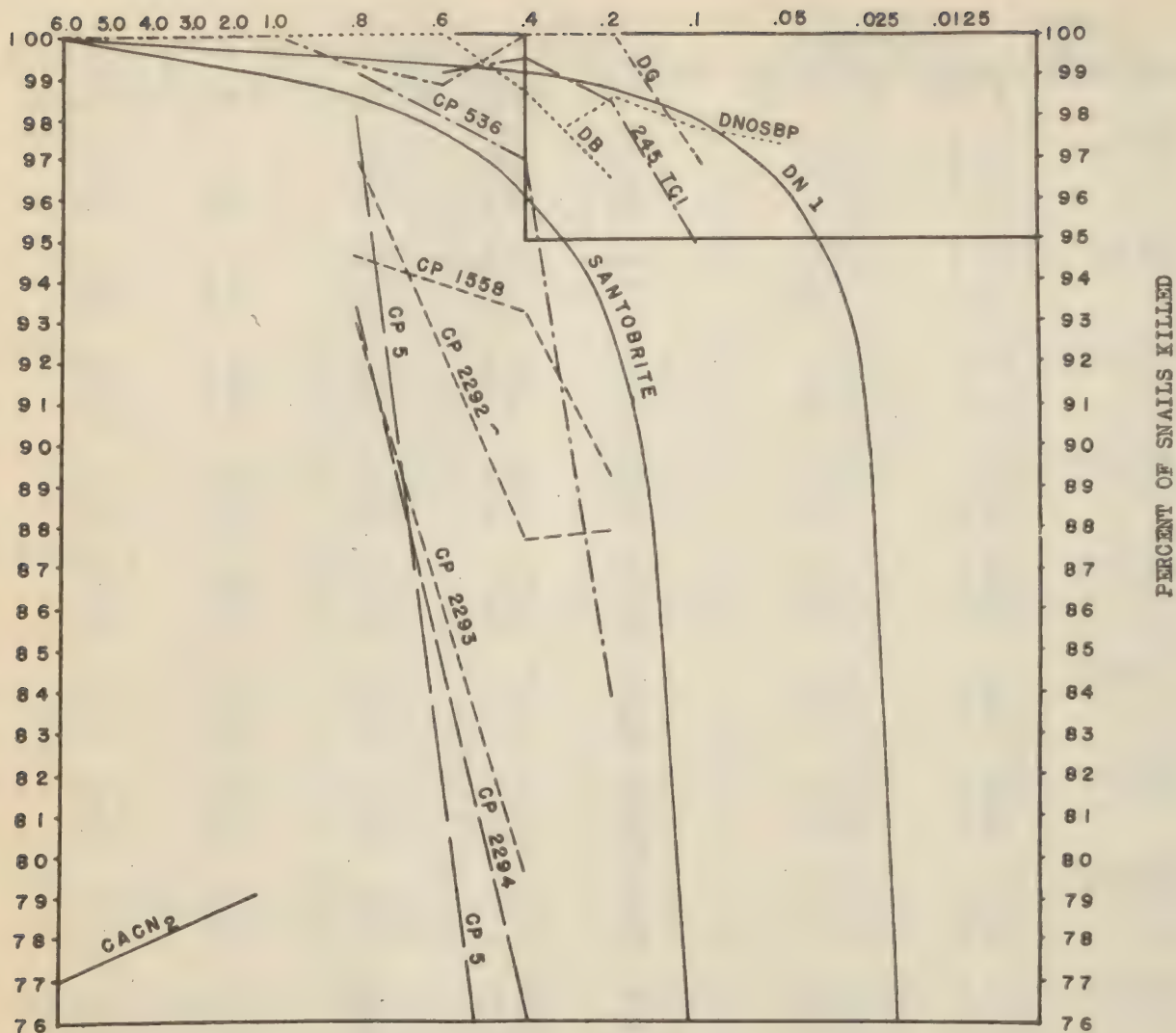


Figure 1. Results of Routine Field-Plot Dilution Tests During Spring, 1952

Key for Graph

- Santobrite - Sodium pentachlorophenate (theoretical curve)
- DG - Sodium pentachlorophenate
- DNOSBP - Dinitro-o-sec. butylphenol
- 2,4,5 TC - 2,4,5 Trichlorophenol
- DB - Sodium 2,4,5 trichlorophenate
- DN-1 - Dinitro-o-cyclohexyl phenol (theoretical curve)
- CaCN₂ - Calcium cyanamide (lime nitrogen)
- CP 5 - 2,3,4,6 Tetrachlorophenol
- CP 2293 - 3-Isocoronylamino propionitrile pentachlorophenate
- CP 536 - 2, 2' Methylenebis (4,6-dichlorophenol)
- CP 2294 - 3-Butylamino propionitrile pentachlorophenate
- CP 2292 - 3-Ethylamino propionitrile pentachlorophenate
- CP 1558 - 1, 3-Diphenylguanidine pentachlorophenate

Table VII. Table of Results of Fall (1952) Field-Plot Dilution Tests

Chemicals	Pre-Treatment Samples (Controls)		Dosage*** Gms/sq ft	Post-treatment Samples			
	% Dead	No. Snails		4 Days		14 Days	
Santobrite	1.5	(204)	0.6	88.1	(143)	89.0	(172)
	0.0	(142)	*	67.3	(205)	67.3	(165)
	0.4	(245)	0.4	91.7	(157)	90.5	(201)
	0.7	(128)	*	59.5	(200)	48.9	(141)
	2.7	(222)	0.2	97.2	(214)	96.6	(238)
	1.5	(142)	*	56.5	(138)	46.8	(136)
	2.1	(243)	0.1	95.2	(147)	75.9	(137)
	0.7	(145)	*	34.0	(141)	33.7	(184)
	1.7	(294)	0.05	56.3	(142)	54.4	(171)
	2.5	(116)	*	18.9	(148)	13.7	(117)
DN-1	0.5	(197)	0.2	95.5	(220)	99.5	(216)
	0.0	(179)	*	82.7	(179)	85.1	(195)
	0.5	(211)	0.1	83.2	(113)	91.8	(171)
	3.6	(152)	*	88.8	(161)	50.6	(269)
	0.9	(213)	0.05	91.7	(168)	88.6	(175)
	1.0	(210)	*	75.1	(221)	63.2	(204)
	2.2	(229)	0.025	85.0	(147)	71.5	(186)
	0.0	(164)	*	89.8	(225)	56.5	(214)
	16.2	(260)	0.0125	85.9	(149)	69.0	(171)
	2.3	(352)	*	58.0	(220)	32.5	(243)
Dowcide-B	0.0	(268)	0.6	99.4	(166)	99.4	(334)
	0.0	(247)	0.4	98.7	(157)	100.0	(228)
	0.5	(222)	0.2	93.2	(176)	91.4	(220)
	0.8	(247)	0.1	52.3	(149)	38.2	(199)
	3.3	(209)	0.05	13.9	(266)	25.8	(190)
	0.0	(212)	0.025	10.3	(165)	7.8	(204)
Dowcide G	7.1	(212)	0.4	60.1	(158)	53.0	(232)
	0.0	(130)	*	85.9	(206)	83.9	(168)
	12.2	(237)	0.2	43.2	(185)	67.2	(186)
	0.0	(183)	*	79.3	(183)	78.8	(222)
	0.9	(323)	0.1	65.7	(137)	27.9	(229)
	9.9	(172)	*	57.3	(185)	61.5	(190)
	1.4	(217)	0.05	70.8	(325)	79.7	(202)
	9.1	(175)	*	49.3	(223)	25.5	(220)
	0.2	(429)	0.025	48.4	(153)	23.2	(272)
	0.8	(113)	*	52.6	(209)	56.0	(166)
DN-2	0.5	(194)	0.8	95.8	(120)	100.0	(182)
	0.0	(224)	0.4	99.4	(170)	97.4	(152)
	0.5	(222)	0.2	85.0	(160)	84.7	(203)
	0.0	(216)	0.1	39.4	(198)	66.5	(185)
	1.0	(197)	0.05	41.7	(240)	77.2	(184)
	0.0	(242)	0.025	16.2	(173)	14.6	(233)
	0.0	(204)	0.0125	28.6	(126)	29.7	(155)

* Second series. Dates of application for first and second series were 9 and 16 October.

** The several entries under each chemical represent individual plot tests.

*** Active ingredient.

the mortality after four days was very low, reaching a high level after fourteen days. DN-1 gave a high kill after four days in both situations.

Inferior and inconsistent results characterized the four-day collections of the second fall series of applications, even though conditions were seemingly more favorable than for the first series. The differences were very likely due to lower temperatures and increased hibernation; dates of application were 9 and 16 October. This conclusion is consistent with previous work (5, 6, 7, 8). DN-1 seemed less affected than Santobrite and Dowcide G. The poorer results for the 14-day post-treatment collections of the second fall series as compared with those of the 4-day collections were undoubtedly due to the fact that irrigation water was inadvertently turned into the ditch between these two collections. Empty shells may have been floated away or covered.

Because of the very irregular results obtained in the fall tests, it is now recognized that April and May are more suitable for field-plot dilution tests. Interference from environmental conditions and farming activities are less likely; and since snail control programs are carried out by the Japanese at this time of year, the environmental conditions for the tests would be the same as would be encountered in the control work.

Figure 1 shows the relative efficiency of the various chemicals. The upper right-hand portion of the graph is critical. For a curve to pass through it, the chemical must give a 95% kill with 0.4 gms per sq. ft. or less. Molluscicides meeting these criteria are designated as highly effective. Those killing at a rate of 90% at this concentration are classed as fair, and below 90% as poor. Of the 21 chemicals tested, 8 may be listed as highly effective.

Santobrite and DN-1 have probably been the most extensively tested of all of the newer molluscicides. These, then, should serve as controls and as a basis of comparison for testing other chemicals. From the spring data, 0.05 gm of DN-1 per sq. ft. was as effective as 0.2 gm of Santobrite. In the fall tests 0.0125 gm of DN-1 was more effective than 0.05 gm of Santobrite, while 0.1 gm of the latter was only slightly better than 0.025 gm of DN-1; and correspondingly, the same ratio was maintained for the next two higher dosages. It therefore appears that the ratio of effectiveness is 4:1 in favor of DN-1. McMullen et al ⁽⁵⁾ obtained similar results with these two formulations. The same effective ratio of 4:1 was obtained in the immersion tests; (see Molluscicide Studies-Laboratory Screening Tests, *supra* vide). The excellent results obtained by Hunter et al ⁽⁶⁾ with Santobrite was accomplished with approximately 0.4 gm per sq. ft. Whether equal results can be obtained with a lesser dosage is conjectural. Final evaluation of these two molluscicides may well have to await further large-scale control efforts employing both.

Dowcide G gave consistently higher kills than Santobrite in the spring tests. This had previously been noted ⁽⁴⁾, but in the fall tests, reciprocal results were obtained.

The consistent results obtained with Dowcide B in both the spring and fall tests indicate that this chemical is highly effective at 0.2 gm per sq. ft. but ineffective at lower dilutions under prevailing conditions. There are indications that 2, 4, 5 trichlorophenol is more effective than its sodium salt (Dowcide B), 0.1 gm giving a kill of 95%.

Dinitro-o-sec-butylphenol gave superior results at all applications, from .3 gm down to .05 gm, over all of the eight good molluscicides. In all dilutions a mortality rate of 97% or higher was noted. The possibility of it surpassing both DN-1 and Santobrite in effectiveness is anticipated in the forthcoming spring tests.

The formulation DN-2 (Dinitro-o-cyclohexyl creosol), was tested only in the fall. The results were promising, since it gave kills comparable to Dowcide B and possessed a good residual quality. Further tests are indicated before any conclusions can be made. Likewise, 2, 2', methylenebis (4, 6 dichlorophenol) gave good results, but requires further testing. Curiously, this chemical was relatively ineffective in the plate tests conducted in the laboratory.

Six other chemicals gave fair results and may warrant further testing, but it seems unlikely that their results will surpass the eight discussed above.

It should be emphasized that with the exception of Santobrite and DN-1, the cost efficiency ratio was not considered in the judgment of an effective molluscicide. When prices are made available, chemicals requiring greater concentrations may still be more economical. Of importance in this regard are the observations gained from the field-plot dilution tests showing that equally as good results may be obtainable with lesser chemical quantities than those indicated by previously conducted field screening tests and large field tests.

MOLLUSCACIDE STUDIES - EVALUATION OF FIELD PROCEDURES: Several methods of applying molluscicides were evaluated, including the use of impounded waters for exposing snails and introduction of chemicals into continuously flowing water. The garden-sprinkler method of applying water-mixed compounds served as a standard, or control test. Consideration was given to the optimum amount of water required as a dispersing medium, and a new method of sampling the snail population was tested.

Procedures and Observations - Application of Chemicals to Impounded Water - Ditches were selected in which water could be readily impounded, including one which was rock-lined with deep crevices. The volume of water introduced was determined, and an appropriate quantity of chemical was thoroughly mixed with it. The mixture was splashed and poured on to the banks and shoulders of the ditches.

Mortality rates for pre-and post-treatment collections are shown in Table VIII. Effective kills of 95% or higher occurred in all four tests undertaken. In one ditch no repopulation was evident at the end of the first summer following treatment. The impounding method, where feasible, has considerable advantage for rock-lined and otherwise irregular ditches, assuring that the chemical reaches all interstices. Grass-cutting and water-carrying are avoided, reducing labor required, but the method appears less economical in the amount of chemical required. In three of the experiments, the concentration of the chemical (DN-1) was calculated at 27, 33, and 39 ppm. The amounts required to give these concentrations were about equivalent to 0.4 gm per sq. ft. This is at least four times the amount required to give equal results with the sprinkler method. However, a test carried out during the fall months with 10 ppm (0.13 gm/sq. ft.) gave equally effective results. It is not anticipated that application of chemicals to impounded water could be used to the exclusion of sprinkling.

Continuous-Flow Application of Chemicals - A continuous-flow application was tried in a ditch 2700 feet in length (Table VIII). The chemical mixed in water at a high concentration was syphoned into the irrigation ditch, where the flow of water was maintained at a depth of 4 to 6 inches. As the mixture was carried down stream, it was splashed and poured by long-handled, night-soil dippers onto the sides and shoulders of the ditch. The amount of chemical thus applied was about 0.2 grams per sq. ft., which at least doubled that required by the sprinkling method. This method is commonly used in Yamanashi Prefecture for the application of calcium cyanamide. Although the average percent of kill (90.4%) was not as high as that achieved by the sprinkler method, certain attributes seem to warrant further tests. Considerably less labor is required as water-carrying is eliminated and cutting of vegetation is not necessary. The pouring and splashing of the water onto the bank and shoulder gave a more thorough coverage than sprinkling, allowing the chemical to penetrate root masses, crevices and holes where snails are commonly located in hibernation or during dry periods. The distribution, however, was not uniform because an excess of the chemical was deposited on the bottom of the ditch. The splashing and pouring of the water tended to offset this since many of the snails were washed from the sides to the bottom of the ditch, thereby increasing the chance of lethal exposure.

The possibility is entertained of a molluscicide killing eggs and newly hatched snails at such low concentrations that it would be practical to apply the chemical directly to irrigation waters at critical times during the summer. If such is the case, the continuous-flow method would be applicable and practical. Experimental

tests carried out at an agricultural experiment station in Kofu have shown that DN-1 and Santobrite at low concentrations do not damage rice plants.

Table VIII. Evaluation of Methods of Applying Molluscacides

Chemical	Ditch Length	Dosage* ppm or gms/sq ft	Molluscacidal Effectiveness**		
			Pre-treatment (Controls)	Post-treatment 4 days	Post-treatment 14 days
SPRINKLING					
DN - 1	30 ft	.2 gm	2.2% (326)	95.4% (287)	96.4% (282)
DN - 1	30 ft	.1 gm	0.9% (206)	89.3% (254)	95.2% (340)
DN - 1	765 ft	0.1 gm	12.0% (2572)	77.0% (2326)	91.7% (1800)
IMPOUNDED WATER					
DN - 1	81 ft	27 ppm .4 gm	4.2% (571)	99.5% (856)	-
DN - 1	175 ft	39 ppm .4 gm	4.3% (451)	91.3% (1541)	96.2% (911)
DN - 1	675 ft	.33 gm	5.6% (2591)	95.8% (3621)	-
DN - 1	350 ft	10 ppm .1 gm	1.0% (556)	85.8% (330)	94.6% (205)
CONTINUOUS-FLOW APPLICATION					
DN - 1	2700 ft	31 ppm .2 gm	4.2% (3105)	87.9% (2000)	90.4% (3086)

* Dosage in all cases refers to active ingredient

** Percent of snails dead and number collected

Molluscicide Efficiency in Relation to Varying Amounts of Diluent Used In Application - It had been suspected that the amount of water used in dispersing the chemicals is a critical factor in molluscacidal effectiveness. As 100 ml per sq. ft. was recognized as about the minimum which would give complete coverage, its effectiveness was compared with 200 ml per sq. ft. This variable was tested by applying 0.1 and 0.2 grams each of Santobrite and DN-1. The differences obtained were inconsequential.

The Sprinkler Method For Applying Molluscacidal Chemicals - The results of this method of application have been thoroughly discussed by earlier workers who found it preferable for general routine application of molluscacides in ditches which were not rock lined or irregular in contour. This method gave the highest percentage

of kill and allowed for better control of the various concentrations of the chemical. Probably the greatest drawback in its use is the necessity of having large numbers of field hands present to cut grass, carry water, and apply the chemical to the ditch. For routine field-plot tests of new chemicals, the method is highly preferable because of its consistent results.

A New Method of Taking Pre- and Post-Treatment Samples of Snail Population -
For the amphibious snail, O. nosophora, the population sampling method previously used entailed collecting all snails in a series of quadrates one foot square. Some such unit of sampling is imperative when population reduction is the criterion for evaluation. In experiments of short duration (two weeks or less), such as routine field-plot tests, the mortality rate is deemed an adequate evaluation method. In such cases an arbitrary number of snails (200) are collected uniformly from the experimental plot. In experiments which must be followed over a long period of time, it becomes necessary to use population reduction as the criterion, since the shells are either soon covered, washed away, or destroyed.

The quadrate method of sampling is not entirely adequate, and a new procedure based on snail numbers collected during a unit of time was tried instead of a unit area. This method was tested in relation to the 2700 foot ditch which was treated by the continuous-flow method of application. The ditch was divided into nine 300-foot sections. Two men collected from each for a period of fifteen minutes. Each covered half the distance, collecting at a series of points throughout the allotted footage. At any one point picking proceeded from the bottom of the ditch to the shoulders, covering a variable width as dictated by the snail density. By the procedure outlined eight men collected about 3,000 snails in 32 minutes. It is imperative that the same individual be used for both pre- and post-treatment collecting. The method is believed to have considerable merit.

THE INCUBATION PERIOD OF THE EGGS OF ONCOMELANIA NOSOPHORA: Original observations on egg-laying and incubation for O. nosophora were made by Sugiura (9), followed by Abbott (10) and McMullen (11) on O. quadrasi. The first two investigators reported a 14-15, and a 15-day incubation period, respectively. From our 1951 observations (4, 12) initial hatching occurred on the twelfth day after laying, but the process was staggered, ranging up to thirty-five days. When the data were graphically represented, two peaks of hatching at 16-19 days and 24-27 days were noted - a bimodal curve.

Two additional experiments on incubation time of O. nosophora eggs were made in 1952, one during March and April at temperatures of 20-25°C. and the other during May and June at temperatures ranging from 25-29°C.

Procedures - Female snails, distinguished on the basis of size, were maintained on moist filter paper in petri dishes (1) with decayed leaves and straw as food. Since it had been noted in 1951 (4) that snails in petri dishes showed a preference for soil as a laying site, in contrast to leaves, straw, and filter paper, small mud cubes were placed in each dish. These were prepared simply by packing mud in a petri dish, and after partial drying, cutting it into one-half inch cubes. The eggs were laid singly in the mud in shallow excavations, prepared by the snail. The depression appeared to be lined and in turn capped with a fine-textured material. If laid on leaves, straw or filter paper, the eggs were covered with a dome-shaped case. All mud cubes containing eggs, and isolated egg-cases located elsewhere in the dish, were transferred daily from the series of laying chambers to a single petri dish, which served as an incubation chamber. Separate incubation chambers were used for each day's collection. Mud cubes were replaced for subsequent collection. When hatching started, the young snails were removed from the incubation chambers daily.

The first group of 383 eggs was collected between 10-31 March and the second of 1098 eggs from 15 May to 11 June. Each group was actually comprised of a series of collections, incubation being timed for each collection individually.

Results - Data on incubation time of *O. nosophora* eggs are graphically represented in Figure 2. A few eggs hatched as soon as 11-13 days after deposition, but the majority required longer, ranging to 36 days. This range was the same for those laid in March as those in May and June. The period of maximum hatching, however, differed for the two groups, occurring 24-25 days after laying in case of the earlier collections and 18-19 days for those deposited in May and June. This difference is undoubtedly associated with a temperature variable. Daily temperature readings gave a range from 20° - 25°C for the early group and 24° - 29° for the later one.

The observations of Sugiura (9) and Abbott (10) coincide only with the initial portion of the time-range noted in the current study. Although the conditions of the petri dishes appeared quite favorable for laying and incubation, as indicated by the number of eggs collected and a hatching rate of about 85%, it is possible that they did not afford optimum conditions. It is still unknown whether the snail lays primarily on moist surfaces in nature or has a preference for water. If eggs are normally deposited on moist surfaces, it is possible that they subsequently become submerged.

The initial observations in 1951 (4, 12) suggested two peaks of hatching, but this was not confirmed in 1952. It is believed that favorable hatching conditions were unknowingly interrupted in the 1951 experiment; possibly the moisture of the dishes fell below the optimum.

The possibility of snail control measures being expedited by knowledge of egg-laying and incubation is entertained. Marked lethal effects of molluscacides on newly hatched snails at very low concentrations has been demonstrated in the laboratory. Molluscacides successfully introduced in irrigation water within a month after maximum egg laying, and repeated every two or three weeks during the egg-laying period, might result in effective control.

EGG LAYING TENDENCIES OF ONCOMELANIA NOSOPHORA: Only limited investigations have been made on the egg-laying habits of *Oncomelania nosophora*. The eggs were first observed by Sugiura (9), who recovered them initially from the natural environment and subsequently under laboratory conditions. His observations included those on the seasonal duration of laying and the number of eggs produced per female. These same points of consideration were recently studied by Ishii and Tsuda (13).

During the spring and summer of 1952, several aspects of oviposition for *O. nosophora* were investigated, including the occurrence of laying in correlation with snail-size; the seasonal duration of reproduction; and the duration of fertility after copulation.

Methods - Female snails, distinguishable in part on the basis of a size differential, were maintained on moist filter paper in petri dishes containing mud cubes and decayed leaves, and straw included as food. As it was difficult to recognize and count the eggs in the mud, the reproduction capacity was determined on the basis of newly-hatched snails. It was estimated that 85% of the eggs hatched.

Observations and Discussion - Occurrence of Laying and the Reproductive Capacity Correlated with Snail Size - The data collected are incorporated in Table IX. A total of 113 female snails were individually isolated and observed for laying. Of these, 100 (89%) laid eggs from which young snails hatched. The occurrence of laying did not vary for snails measuring 7.0, 7.5, and 8 mm in size. The single specimen measuring 6.5 mm laid, while 12 measuring from 5.0 to 6.0 mm did not.

The average number of young produced by each female was 23. The variations in this figure for the several snail sizes were hardly significant. Eight of 53 females produced 10 or less offspring; three accounted for 50 or more, the largest number being 65, while 32 produced between 11 and 30 young.

For several groups of snails under different laboratory conditions Ishii and Tsuda (13) noted variations in egg-production ranging from 17 to 100 eggs per female.

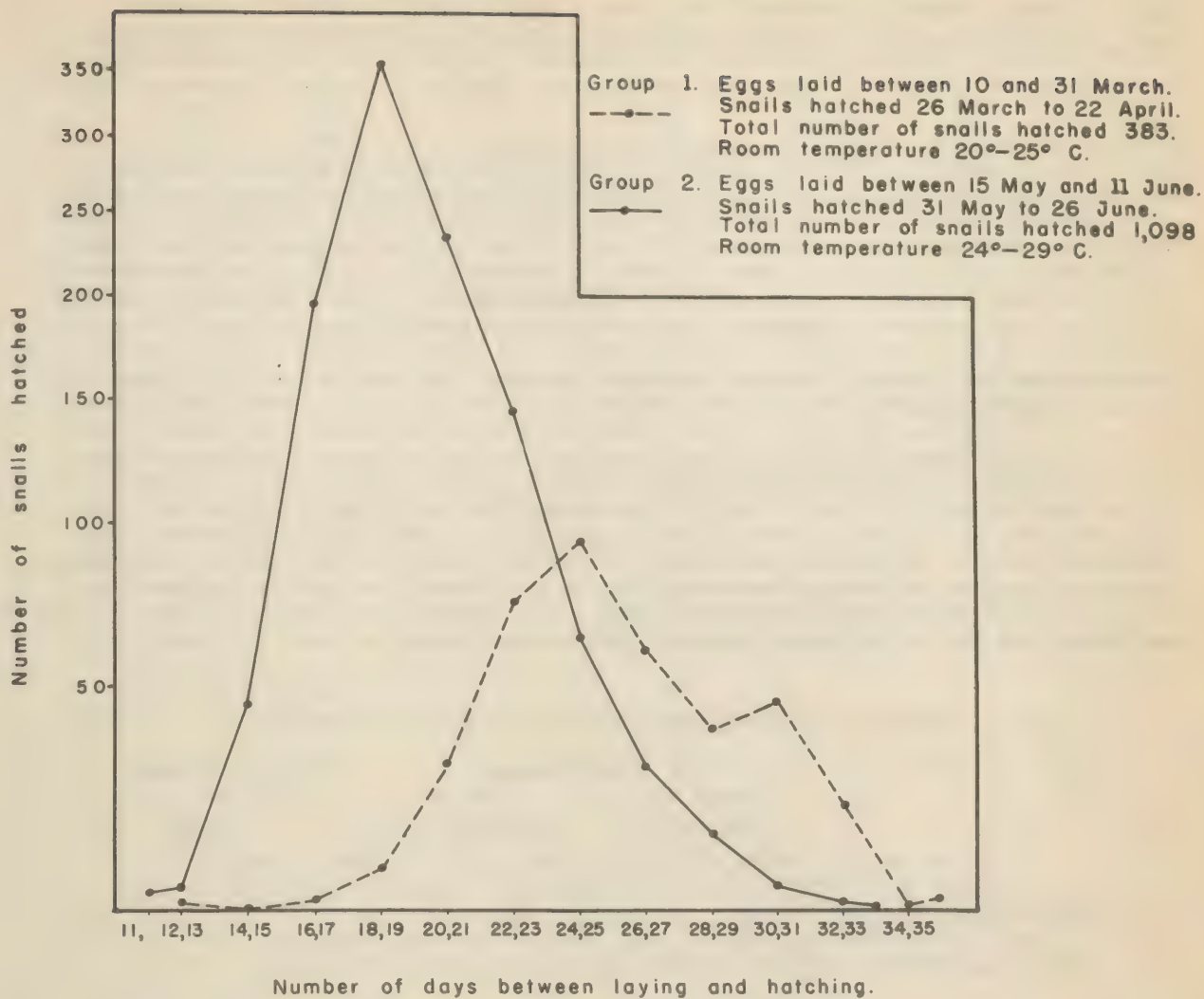


Figure 2. Incubation Period of Oncomelania nosophora Eggs

Table IX. Reproduction of Females Correlated with Snail Size

Snail Size (mm)	Occurrence of Laying			Number Offspring Produced		
	No. Females Observed	Number Laying	Percent Laying	No. Females Observed	Number Young	Average Young
8.0+	45	40	89%	20	512	26
7.5	39	35	90%	26	569	22
7.0	29	25	86%	6	108	18
6.5	1	1	-	1	32	32
5.0-6.0	12	0	0%			
Totals*	113	100	89%	52	1189	23

* Only snails 7.0 mm and over

They believed that the size of the containers in which the snails were maintained exerted a significant influence on reproduction.

The Seasonal Duration of Reproduction - Snails collected about mid-February were maintained at 10°C until early March, when they were individually isolated on filter paper in petri dishes. Initial laying was noted on 10 March, which was probably about two months prior to the first laying in the field. This difference was undoubtedly due to the higher temperature of the laboratory (about 20°C). Two experimental categories of females were established; (a) those individually isolated, and (b) those isolated in pairs, male and female. Considering first the total figure, it was found that 44% of the young were hatched in April (none in March), (Table X).

Table X. Monthly Recovery of Newly-Hatched Snails

	Snail Groups					
	Males Not		Males		Totals	
	Included		Included (1:1)			
	No.	%	No.	%	No.	%
No. Females Laying*	15		38		53	
Snails Hatched In:						
April	208	50	329	40	537	44
May	59	14	120	15	179	15
June	76	18	125	15	201	16
July	58	14	149	18	207	17
August	<u>13</u>	<u>3</u>	<u>92</u>	<u>11</u>	<u>105</u>	<u>9</u>
Total	414	100	815	100	1229	100

Av. Young/Female 27.6 21.4

* All males were finally crushed, thus priving their sex.

During the months of May, June, and July hatching was at a uniform level, the respective figures being 15, 16, and 17%. Only 9% of the total were hatched in August, and none in September. It was noted that the females producing the most eggs had the longest laying span.

The inclusion of a male with each female did not result in an increase in the number of young produced. Although copulation was noted, records were not kept of the extent. The data did suggest that a larger portion of the females brought in from the field in April laid than those collected in February. Regardless, the observations

warrant the conclusion that a high level of fertility is assured even prior to the spring peak of copulation.

In contrast to our findings Ishii and Tsuda (13) observed initial laying in May with a peak in June and July. Their observations are to be expected in nature. Very likely the temperatures of their laboratory were low during the spring months. In their experiment, laying continued throughout August, but at a very low level.

Duration of Fertility Following Copulation - A group of eight female snails originally collected in copulation during the spring of 1951, were maintained in isolation continuously until the spring of 1952. All had laid in the interim. In March 1952, each of four received two males, while the other four were retained in isolation. Egg-laying occurred in March in both groups and newly-hatched snails were recovered in April. The numbers of young produced by the two groups were about equal. In May, however, the females having received males produced a much larger number of young. This difference was maintained throughout June and July, with only a moderate drop in August (Table XI). Females which did not receive a male in the spring of 1952 produced a small number of young. The numbers of offspring per female for the two groups for each month is shown in Table XI. It is highly significant that females can produce viable eggs for more than one year after copulation.

Table XI. Duration of Fertility Following Copulation

	Number Females Isolated	Newly Hatched Snails Recovered in 1952					
		April	May	June	July	Aug.	Average*
Males not Included in 1952	4	15	9	9	4	0	9.3
Males** Included in 1952	4	18	49	36	39	25	41.8

* Average number of eggs laid in 1951 by the 8 females was 15.6

** Males were crushed at the end of the experiment to prove their sex.

In three dishes both were males. In one dish one of the specimens believed to be male was, in fact, a female, but size and body condition made it unlikely that it had laid.

A single fertilized female remaining after molluscicide application could account for re-population even in the absence of a male. From field observation in Kyushu (14) the residue of snails following four applications of Santobrite was found to be about 95% female. These two observations constitute a serious barrier for control measures directed at adults. Control measures should include attack at eggs and newly hatched snails as well.

PROSPECTUS FOR CONTROL MEASURES AGAINST NEWLY-HATCHED ONCOMELANIA NOSOPHORA:

The circumstances and possibilities pertaining to control of *O. nosophora* by attacking the egg and newly-hatched stages of the snail are being given critical consideration. The very young snail is, in contrast to the adult, apparently limited to the aquatic habitat. This, and the absence of a protective shell, make it vulnerable to chemicals introduced into irrigation water. The chief problem in this regard is whether the MLD for newly-hatched snails is sufficiently low to make dispersal in the rapidly-flowing irrigation waters economically practical. Only preliminary information (Table XII) is available. Both Santobrite and DN-1 were applied to newly-hatched snails at concentrations of 1.0, 0.5, and 0.25 ppm (active ingredient).

Table XIII. The Effects of Very Low Concentrations of Santobrite and DN-1 on Newly-Hatched O. nosophora

Conditions of Experiment	Observations After Varying Intervals of Exposure			
	30 minutes	2 hours	4½ hours	6 hours
Controls	All active	All active	12 active 1 dead 2 lost 1 closed	9 active 1 dead 2 lost 4 closed
DN-1 1.0 ppm.	All active	1 active 7 closed	1 closed 7 no reaction	8 dead
DN-1 0.5 ppm.	All active	8 closed	1 closed 7 no reaction	1 no reaction 7 dead
DN-1 0.25 ppm.	Some closed	8 closed	8 no reaction	1 no reaction 7 dead
Santobrite 1.0 ppm.	All active	1 active 5 closed 2 no reaction	5 closed 3 no reaction	1 no reaction 7 dead
Santobrite 0.5 ppm.	All active	1 active 6 closed 1 no reaction	3 closed 5 no reaction	8 dead
Santobrite 0.25 ppm.	All active	3 active 5 closed	6 closed 2 no reaction	2 no reaction 6 dead

16 snails used in control group

8 snails used in each of the other groups

Closed - operculum closed, contracted when operculum was touched

No reaction - operculum closed and no reaction to touch

Dead - body distended from shell, no response to touch

Initial damage was evident with both molluscicides after two hours in all three concentrations; after 4½ hours, they were either dead or dying and after 6 hours, most of the young snails were considered dead. It appears, then, with a six-hour exposure period that the MLD for both Santobrite and DN-1 will prove to be less than 0.25 ppm. Attempts to confirm these findings will be made during the spring of 1953. If they prove correct, it will be of interest to compare the susceptibility of eggs and newly-hatched snails to molluscicides. Kuntz and Wells (7), limiting attention primarily to eggs of Biomphalaria and Bulinus, found they were killed by 1-3 parts of dinitro-o-cyclohexyl-phenol (active ingredient of DN-1) in 24 hours. The protection of the gelatinous layers of the egg against molluscicides may prove to be considerable, making the newly-hatched a more favorable stage to attack. It is still not known whether the eggs of O. nosophora are primarily laid in water, or on the moist surfaces immediately above the water line where they would be less vulnerable to molluscicides.

IMMUNOLOGIC RESISTANCE AGAINST SCHISTOSOMA JAPONICUM IN THE MAMMALIAN HOST AS DEMONSTRATED BY REINFECTION: We have previously made attempts to demonstrate immunologic resistance by repeated infections in hamsters and mice. Two experiments have been completed (4) and a third is here reported. The preceding experiments suggested that some resistance is derived from initial immunizing exposures.

Results -(Table XIII) A total of 130 adult worms (average 6.8) were recovered from 19 control mice, each of which received ten cercariae on the initial exposure. Twenty-four reinfection controls receiving 80 cercariae each, harbored 946 worms, or an average of 39.4 worms per animal. For these two groups, the recovery rates of cercariae as adults were 68.4 and 49.3 respectively. The average rate of 51.0% was consistent with results obtained in this laboratory over a period of 4-5 years.

Table XIII. Immunologic Resistance Against Schistosoma japonicum as Demonstrated by Reinfection

	Control Animals		Totals	Experimental Animals
	For Initial Exposures	For Reinfections		
No. Mice Infected	40	40		40
Date of Exposure	22-24 April 1952	24 July to 2 August 1952		22-24 April 1952 and 24 July to 2 August, 1952
Date of Autopsy	After 1 month	After 1 month		1 month after second exposure
No. Cerc./Mouse	10	80	90	90
No. Mice Posted	19	24		28
Worms Recovered	130	946	1076	761
Av. Worms/Mouse	6.8	39.4	46.2*	27.2
Recovery Rate of Cerc. as worms	68.4%	49.3%	51.0%	30.2%
Reduction of Worm Burden				41.1%

* This figure is the sum of the average worm recoveries and serves as a basis (100%) in determining the percentage of reduction in worm burden.

Each of the 28 mice under study received 10 cercariae for the immunizing exposure and 80 at the time of reinfection. They were exposed, then, to the same number of cercariae as those of the control groups combined. The recovery of 761 worms from the 28 experimental mice represents an average of 27.2 worms per mouse or a recovery rate of 30.2%. This constitutes a reduction in worm burden of 41.6%. It may be noted again that the recovery rate of cercariae as adults in animal infections has been quite consistent in this laboratory over a period of several years. In light of this, and controlled as the experiment was, it seems that the reduction in the worm burden is significant. The results were in accord with those of the two previous experiments, for which the worm burden reduction for mice was 36.9% and 37.3%, respectively (4). It is believed that evidence of immunologic resistance has been demonstrated.

INVESTIGATIONS ON SCHISTOSOMIASIS IN TAIWAN: The existence of an intermediate molluscan host (Oncomelania formosana) of Schistosoma japonicum in Taiwan has long been known. Although animal reservoirs have been noted, bonafide human infections have never been reported. It has been assumed by some that a careful survey would probably reveal human cases. Dr. H. F. Hsu, Department of Zoology, National Taiwan

University, examined stools of approximately 4,000 persons without conclusively demonstrating schistosome eggs. Single eggs were noted in 2 or 3 cases but none were confirmed. The simple sedimentation technic, which was effectively used, is generally recognized as a satisfactory method for the recovery of eggs. It was proposed that the skin-testing procedure developed in this laboratory be employed to complement the above diagnostic procedure. An invitation was extended to the Chief of the Department to visit Taiwan to perform these tests. The project was accomplished between the 4th and 26th of September.

Approximately 2,500 persons in six villages of the endemic area of central Taiwan were skin-tested with an adult-worm saline extract in a concentration of 1:10,000 (merthiolated). This antigen was prepared from specimens obtained by experimental animal infections. A merthiolate-saline control was also used. A seventh village outside of the endemic area was tested as a control against false positive reactions. A positive reaction was designated as one in which there was an increase in the dermal wheal of 3 millimeters in excess of the control. When enlargements of 2 to 2.5 millimeters occurred, the case was designated as doubtful, and less than this were considered negative.

Positive reactions were obtained in about 7 percent of all the people examined, ranging for the several villages from about 4 to 17 percent. The reactions were quite typical in size and appearance as compared with those encountered in hyper-endemic areas of Japan. In the nonendemic control area, only one person among 140, or 0.7%, tested gave a significant reaction. In this case the wheal was of minimum size and might fairly have been called doubtful. Similar checks in nonendemic areas in Japan have indicated that false positives are infrequent. The antigen has been shown to produce no reaction in cases of paragonimiasis or clonorchiasis.

In other endemic areas of *Schistosoma japonicum* a high percent of the children are infected with a maximum incidence being reached by 15 years of age. In Taiwan, positive reactions did not occur in children under 10 years of age.

Concerted effort will now be made by Dr. Hsu and associates to recover schistosome eggs from the persons found positive by skin-testing. Three different techniques will be employed on 3 stools from each person - the AMS III (acid-sulphate-ether) concentration technic, an egg-hatching method, and the sedimentation method which has been routinely employed.

Even if eggs are finally recovered from humans, it is anticipated the infections will be light and that the disease is not one of public health concern in Taiwan. On the other hand, if eggs are not recovered from any of the positive cases, it is probable that humans are not a suitable host for the Taiwan strain of schistosomiasis. Initial experimental infections of Formosan monkeys carried out by Dr. Hsü have already suggested this, as only immature worms have been recovered. Before final conclusions are reached exposure of human volunteers will be undertaken. In addition to attempting egg recovery it will be determined whether they become positive to the intradermal tests, either with or without egg production. It is believed that Dr. Hsü's investigations, as here outlined, will furnish conclusive information regarding the status of human schistosomiasis in Taiwan.

THE PENETRATION-TIME FOR THE CERCARIAE OF SCHISTOSOMA JAPONICUM: Although innumerable animal infections with *Schistosoma japonicum* have been performed since its discovery by Katsurada, in 1904, the time required for cercariae to gain entry into the mammalian host has not been conclusively demonstrated. Faust (15) mentioned that entry of schistosome cercariae into the epidermis may require less than 10 minutes. Iio (16) investigated this problem with cercariae of *S. japonicum* by means of histopathologic section, while Hitchcock (17) directly observed penetration of the cercariae of *S. mansoni*. Currently, on the basis of worm recovery at autopsy, evidence of a more conclusive nature has been obtained regarding the penetration-time for the cercariae of *S. japonicum*.

Methods - Experimental animals were shaved over most of the back 24 hours before exposure. The method of exposure was that previously reported by Pan et al (18), with the exception that the animals were held by hand. Commonly, 75 to 100 cercariae were used for each infection. Variations in the time allowed for penetration varied from 10 seconds to 5 minutes. Immediately upon completion of exposure, the animals were thoroughly rubbed dry with gauze. They were sacrificed three to four weeks later. The recovery of the adult *S. japonicum* was facilitated by the use of the perfusion technic previously described by Pan and Hunter (19).

An attempt was also made to determine whether the water film and/or evaporation of water adhering to the skin after exposure enhanced penetration. This was done by using short exposure periods (10 and 15 seconds); air-drying under an electric fan, which required less than five minutes; and then comparing the worm recovery with that of the three and five minute exposures which were terminated with immediate drying of the animals.

Results and Discussion (Table XIV) Infections were obtained in mice with periods of exposure as short as 10 seconds, while for hamsters the shortest interval affording infections was 30 seconds. Although 50-75% of the animals became infected for all exposure intervals under three minutes, the percentage of the cercariae recovered as adults remained low, and did not increase progressively, particularly in the mouse series. The few worms taken at autopsy for exposure periods less than three minutes may represent those cercariae protected from wiping by gaining access to skin follicles or crevices, actual penetration of the epidermis occurring later.

When three minutes were allowed for penetration, a striking increase in the infection level occurred; 97.7% of the cercariae applied to hamsters were recovered as adult worms. The number of hamsters used was small, but such results have, not uncommonly, been obtained in previous experiments where 30 minutes were allowed for penetration. When three-minute exposures were used for mice, 52.0% of the cercariae were recovered while from a five minute exposure this figure increased only to 55.2%. Essentially the same results have been obtained consistently in this laboratory for mice with exposures of 30 minutes. It appears, under the conditions of the method used, that three minutes afforded near-maximum infections in both hamsters and mice.

These results are essentially in agreement with work of Hitchcock (17) who observed actual penetration. For 75 cercariae seen entering the skin of hamsters, the average penetration time was 4.3 minutes, the range being 2-15 minutes. For mice, with 50 cercariae observed as penetrating, the corresponding figures were 3.6 minutes and 2.5-8 minutes.

Apparently the method used for exposing the animals (18) tends to foster almost simultaneous contact with the skin by all cercariae. This is reflected by the abrupt occurrence of a near-maximum recovery of cercariae after three-minute exposures. More than 3 to 5 minutes for exposure of mice and hamsters is not necessary, affording a considerable saving in time and lessening damage to the experimental animals. Under natural conditions, such a rapid penetration by cercariae certainly increases their chance of completing the infection cycle. The hazard for the investigator is evident.

Only limited observations were made on the effects of the water film and/or evaporation on penetration (Table XIV). Combining short contacts (10 and 15 seconds) with rapid air-drying (total time not exceeding three-five minutes) recovery percentages of 35.9 and 65.5% were obtained for mice. The effectiveness, or hazard, of even very short contacts with water containing cercariae, followed by air-drying is evident. The recovery rates of 52.0 and 55.2% (mice) for three and five minute exposures, terminated with immediate drying, were exceeded by the 15 second exposure combined with rapid air-drying (65.5%). This suggests some benefit from the water film and/or evaporation. The observation is inconclusive due to the small number of mice used for 15-second exposure. However, a method of determination may have been developed. Such an experiment is not indicated using hamsters, as maximum infections were obtained without air-drying.

Table XIV. Recovery Rates of Cercariae as Adult Schistosomes With Different Exposure Times

Exposure Time	Animals Exposed	Animals Infected	Percent Infected	Total Cercariae	Worms Recovered	Recovery Rate (%)
<u>MICE**</u>						
10 sec.	9	7	77.8	1086	44	4.1
15 sec.	10	5	50.0	1021	90	8.8
20 sec.	10	7	70.0	850	87	10.2
30 sec.	10	7	70.0	674	49	7.3
1 min.	9	5	55.6	394	13	3.3
2 min.	4	3	75.0	199	13	6.5
3 min.	14	13	92.9	727	378	52.0
5 min.	10	10	100.0	446	246	55.2

HAMSTERS**

20 sec.	8	0	0.0	605	0	0.0
30 sec.	6	3	50.0	557	8	1.4
1 min.	8	4	50.0	697	57	8.2
2 min.	5	5	100.0	482	68	14.1
3 min.	3	3	100.0	176	172	97.7

MICE***

10 sec.	10	10	100.0	1037	372	35.9
15 sec.	5	5	100.0	565	370	65.5

- * Animals were wiped dry immediately after exposure
- ** Animals were wiped dry immediately after exposure
- *** Animals quickly air-dried (3-5 min.) by electric fan

THE EPIDEMIOLOGY OF INTESTINAL PROTOZOA IN FAMILY GROUPS: Introduction - The epidemiologic study of intestinal protozoa is a broad undertaking which has been approached from many directions. Consideration here is directed toward the family group as a means of investigating endemic transmission of intestinal protozoa, including the frequency at which new infections occur, and the possibility of spontaneous elimination.

The wealth of information available from such an undertaking has not been widely uncovered either in Japan or in other countries, presumably because of the large physical requirements imposed by such a task. One of the largest such family studies reported was conducted by Seckinger (20) in which he observed familial trends in amebiasis among 69 families. Craig (21) summarized the reports of several investigators who, by examining families where infections were found in one member, reported that other members of these families were also frequently infected with *E. histolytica*. Mackle and Nauss (22) reported on a family of 6 living in New York, four of whom were infected with *E. histolytica*, the probable mode of transmission having been through a parent who had resided outside the city.

The present study is directed toward considering the effect of age, sex, and household duties on incidence of amebiasis, on spontaneous elimination of parasites, and acquisition after treatment. Because of the magnitude of the undertaking the final results of all the above objectives can not be reported at this time.

Methods - In the fall of 1951, a brief survey of towns near Kofu City in Yamanashi Prefecture was conducted for the purpose of selecting sites for the current

epidemiological investigation. Four villages showing high rates of protozoa were selected and have been periodically observed during the past twelve months. Two of these villages, Mutsusawa and Kissawa, are situated on mountain sides while the other two, Tatomi and Hikawa, are found on the flat of the Yamanashi Valley, a triangular area approximately 20 by 18 by 13 miles, enclosed by mountain ranges. Mutsusawa draws its drinking water from the Kamesawa River and a rapidly flowing diverted irrigation ditch which runs through numerous villages above this town and as many more below before joining the large Ara River. The stream conveniently serves as a medium for washing out "honey buckets", washing clothes, catching the spillage and seepage of the latrines, as well as being the source of the drinking water. In Kissawa, the second mountainous town, the drinking water is derived from wells, as is also the case for the two towns on the valley floor. One of the latter, Hikawa, has sometimes been flooded and studies are currently in progress to examine neighboring towns, one more frequently flooded, and one not flooded. The implication between contaminated wells and protozoan infection is evident.

The first series of repeated examinations was conducted in January, 1952 and included almost 1100 individuals. Of these, 1058 submitted a sufficient number of specimens to be included in the "true incidence" analysis. From these 1058 persons, 5229 specimens were submitted, an average of 4.94 per person. Each person was examined once every other day, over a period of ten days. The fecal specimens were brought to the village office on the morning of collection and then were brought by vehicle into Kofu City where the examinations were carried out in the 406th Medical General Laboratory special railroad laboratory car. There, the specimens were prepared and examined by highly skilled technicians using the formalin-ether concentration technic (23). A study of the efficiency of the technic and the technicians is reported elsewhere.

Discussion of Epidemiological Findings. "True" incidence - The results of the overall examinations are shown in Table XV. For the "true" incidence data, only the January examinations from each village were used. Five examinations were carried out over a period of ten days on each individual. The E. histolytica incidence ranged from 27.6% to 13.5% (average 20.6%) in the four villages. Other protozoa rates average 49% E. coli, 34% E. nana, 5.6% I. bütschlii, and 12.3% G. lamblia. It is to be noted that these villages were selected because of their high rates of protozoa and are not, therefore, "average" areas.

Table XV. Incidence of Intestinal Protozoa
(January - 1952)

	Mutsusawa		Tatomi		Hikawa		Kissawa	
	No.	%	No.	%	No.	%	No.	%
No. Examined	323		229		258		248	
No. Specimens	1578		1132		1284		1235	
No. with Protozoa	207	64.1	130	56.2	164	63.6	171	69.0
<u>E. histolytica</u>	89	27.6	31	13.5	43	16.7	55	22.2
<u>E. coli</u>	151	46.7	95	41.5	131	50.8	137	55.2
<u>E. nana</u>	100	31.0	77	33.6	98	38.0	88	35.5
<u>G. lamblia</u>	53	16.4	24	10.5	14	5.4	40	16.1
<u>I. bütschlii</u>	29	9.0	3	1.3	14	5.4	14	5.6

Seasonal Variations - Since individuals in Hikawa and Kissawa were not treated after the January study, a comparison of the January (winter) and September (summer) findings may be made. The data indicate that there was little variation that could be attributed to the season. While there was 2.4% more E. histolytica in January than in September in Hikawa, there was 5% less amebiasis in Kissawa. There were similar variations in the incidences of other protozoa between January and September, but none of the fluctuations were great enough to indicate that seasonal variations are significant.

Acquisition of Intestinal Protozoa Following Treatment - Individuals in Mutsusawa and Tatomi were treated for protozoa after the January examination. Five weeks after treatment (March) individuals were again examined five times each to determine the infection rates. During the following six months new cases of protozoa were acquired as shown in Table XVI.

Table XVI. New Infections With Protozoa After Treatment*

Village: Date:	Mutsusawa				Tatomi			
	March		Sept.		March		Sept.	
	No.	%	No.	%	No.	%	No.	%
No. Persons	319		314		233		223	
With Protozoa	103	32.3	140	44.6	66	28.3	74	33.2
No. Specimens	1536		1546		1144		1093	
<u>E. histolytica</u>	6	1.9	13	4.1	2	0.9	5	2.2
<u>E. coli</u>	48	15.0	57	18.2	29	12.4	38	17.0
<u>E. nana</u>	28	8.8	54	17.2	35	15.0	39	17.5
<u>I. butschlii</u>	1	0.3	3	1.0	1	0.4	1	0.4
<u>G. lamblia</u>	43	13.5	64	20.4	18	7.7	24	10.8

* Treatment given 5 weeks prior to March examination

New infections that appeared during this 6-month period presumably indicate the normal rapidity with which protozoa infect persons in the communities under consideration. It is interesting to note that these increases were comparatively few in number. The value of mass therapy depends to a large degree on the rapidity with which the population becomes reinfected after treatment. These new cases reported in September were probably newly acquired, but might, at least in part, have been treatment failures. In either event, the data indicate that therapy was effective and that reinfection takes place slowly, thus enhancing the value of the therapy.

Spontaneous Elimination of Infections - The data collected over a period of eight months indicate that possible eliminations appeared infrequently. Some such "losses" can be accounted for by the diagnostic limitations and irregularities in submitting specimens. Further observations are being conducted on this matter.

Age Correlated with Rates of Amebiasis - The age of infected persons have been grouped into ten categories, i.e., 0-3, 4-6, 7-10, 11-15, 16-20, 21-30, 31-40, 41-50, 51-60, and over 60. By so doing observations may be made on the rates of infection within each group. These results are summarized in Table XVII.

The fewest number of persons examined in any one age group was 72 (the 51-60 year group), while the largest number fell in the 21-30 year group (151 individuals). The number of positive cases of E. histolytica in each age group is shown in Figure 3. It will be seen that there is a steady increase in the rate of infection through the 51-60 year group. The only major change in the overall infection rate occurs after reaching the age of 60, where there is a sharp decrease from 38.9 to 16.6% infection.

Faust (24) noted for 4,000 patients in New Orleans that the highest incidence occurred between 26-30 years, followed by a rapid decline after 35 years of age. Investigations of Milam and Meleney (25) in Tennessee indicated high levels of infection until 50 years of age, and for older ages only a moderate decrease. Another survey in Tennessee by Meleney, Bishop, and Leathers (26) showed a sharp rise during childhood, a slight increase during middle life, and a decline after 60 years. Culbertson (27) stated that persons over 35 seldom harbor E. histolytica. The current data are essentially consistent with the findings in Tennessee, a sharp progressive increase during childhood and young adulthood, followed by continued though irregular increase during adult life, with a decline after 60 years. It should be noted that the protozoan infections were highly endemic in both the Tennessee area of investigation and Yamanashi. The level of endemicity may well affect the age-incidence

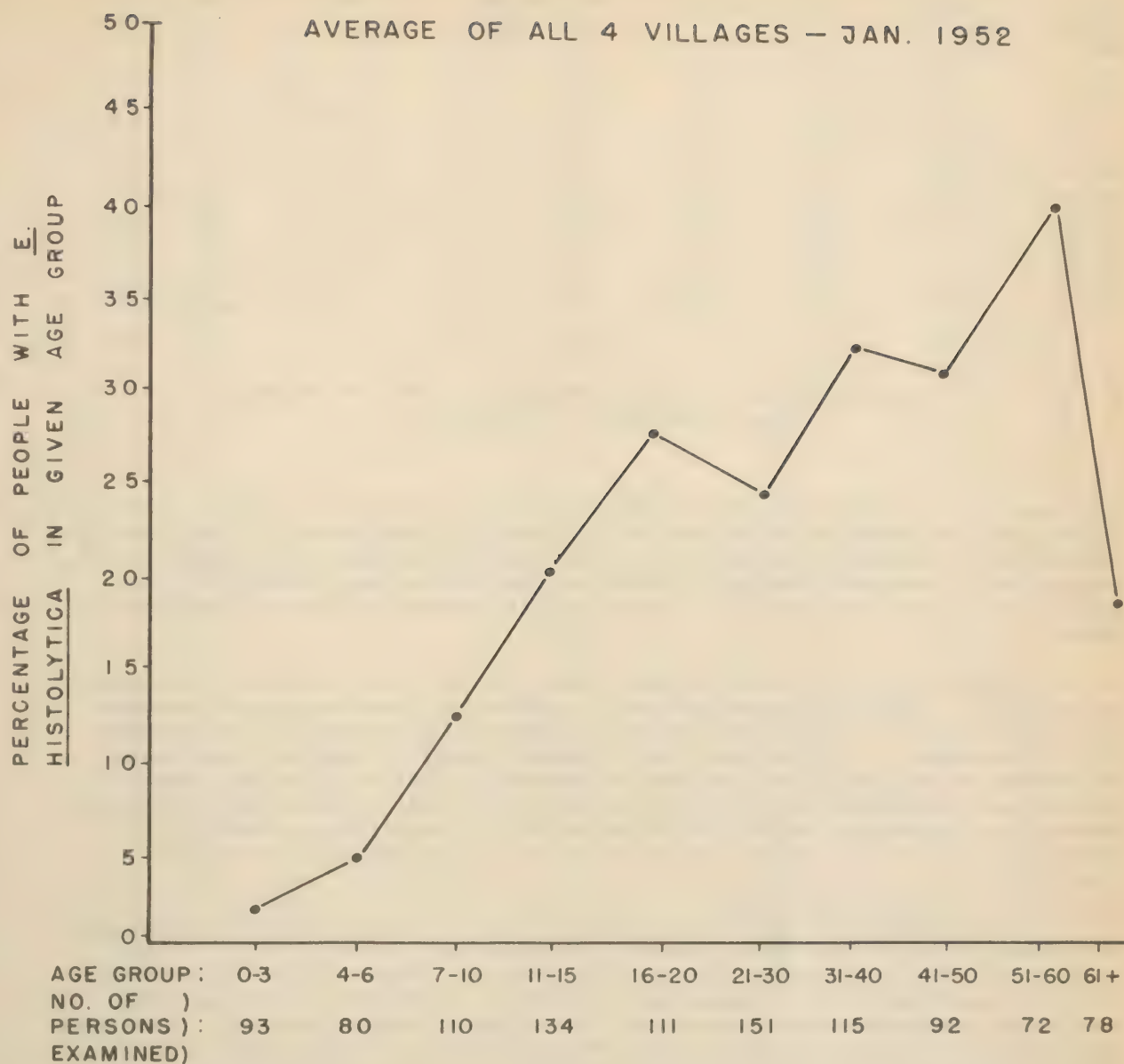


Figure 3. Correlation of Age with Incidence of E. histolytica - 1952

Table XVII. Sex and Age Correlated with Incidence of E. histolytica

Age Group	Total Exam'd.*	Total Positive		No. Examined		Positives			
		No.	%	Male	Female	Male		Female	
				No.	%	No.	%	No.	%
0-3	93	1	1.1	42	51	1	2.4	--	--
4-6	80	4	5.0	44	36	1	2.3	3	8.3
7-10	107	13	12.1	55	52	10	18.1	3	5.8
11-15	134	26	19.4	72	62	9	12.5	17	27.4
16-20	111	30	27.0	55	56	11	20.0	19	33.9
21-30	151	36	23.8	66	85	10	15.1	26	30.6
31-40	115	36	31.3	54	61	16	29.6	20	32.8
41-50	92	28	30.4	53	39	12	22.6	16	41.0
51-60	72	28	38.9	36	36	13	36.1	15	41.7
61+	78	13	16.7	38	40	5	12.5	8	20.0
Totals	1033	215	20.8	515	518	88	17.1	127	24.5

* Correlations based on 5 examinations made in January;
data incomplete for 25 persons

relationship. The observations that have been made possible by the current investigation cast some doubt on the acquisition of immunity to amebiasis which has been postulated by some workers to account for the loss of infections over the age of thirty-five. These data generally tend to confirm the findings of Faust (24) who noted that children under the age of five are less frequently infected than their elders.

The Role of Sex - Sex evidently plays an important role in the incidence of amebiasis. Faust (24) has stated that "...all authorities are agreed that males are more frequently infected than females." Likewise, Culbertson (27) has reported that "In amebiasis ... a disproportionate (higher) number of cases are found among males which is not attributable to greater exposure." The results of our examinations do not corroborate the findings of these other workers. Table XVII summarizes these data. In January, a total of 515 males and 518 females were examined. Of these, 88 males and 127 females were positive for E. histolytica, or a ratio of 1 to 1.44.

In determining the point in life where the rate of female infection exceeds that of the male, Figure 4 was drawn from the appropriate data in Table XVII. The data indicate that the female rate was lower only in the age groups 0-3 and 7-10, where a total of only 11 males and 3 females were concerned. In general, it may be stated that in the area studied the female rate is consistently higher than that of the male. No explanation seems adequate either for the larger number of males infected as reported by other workers, or for the larger number of infected females found in Yamaguchi Prefecture, Japan.

Difficulty of Diagnosing E. histolytica in Small Children - Mention has been made by other workers that difficulty is encountered in diagnosing amebiasis in small children. A study of our data reveals that such seems to be the case. Among children 0-5 years old more cases were diagnosed only once or twice out of five, than in the older groups. Additional examinations are expected to provide further information.

Significance of Food-Handlers in Relation to Transmission of Intestinal Protozoa - Existing information concerning the relative importance of the food-handler in the transmission of intestinal protozoa is not conclusive. Correlations of data (Tables XVIII and XIX) were made to determine whether the food-handler was a primary factor in establishing the high incidence of infection in the population under consideration.

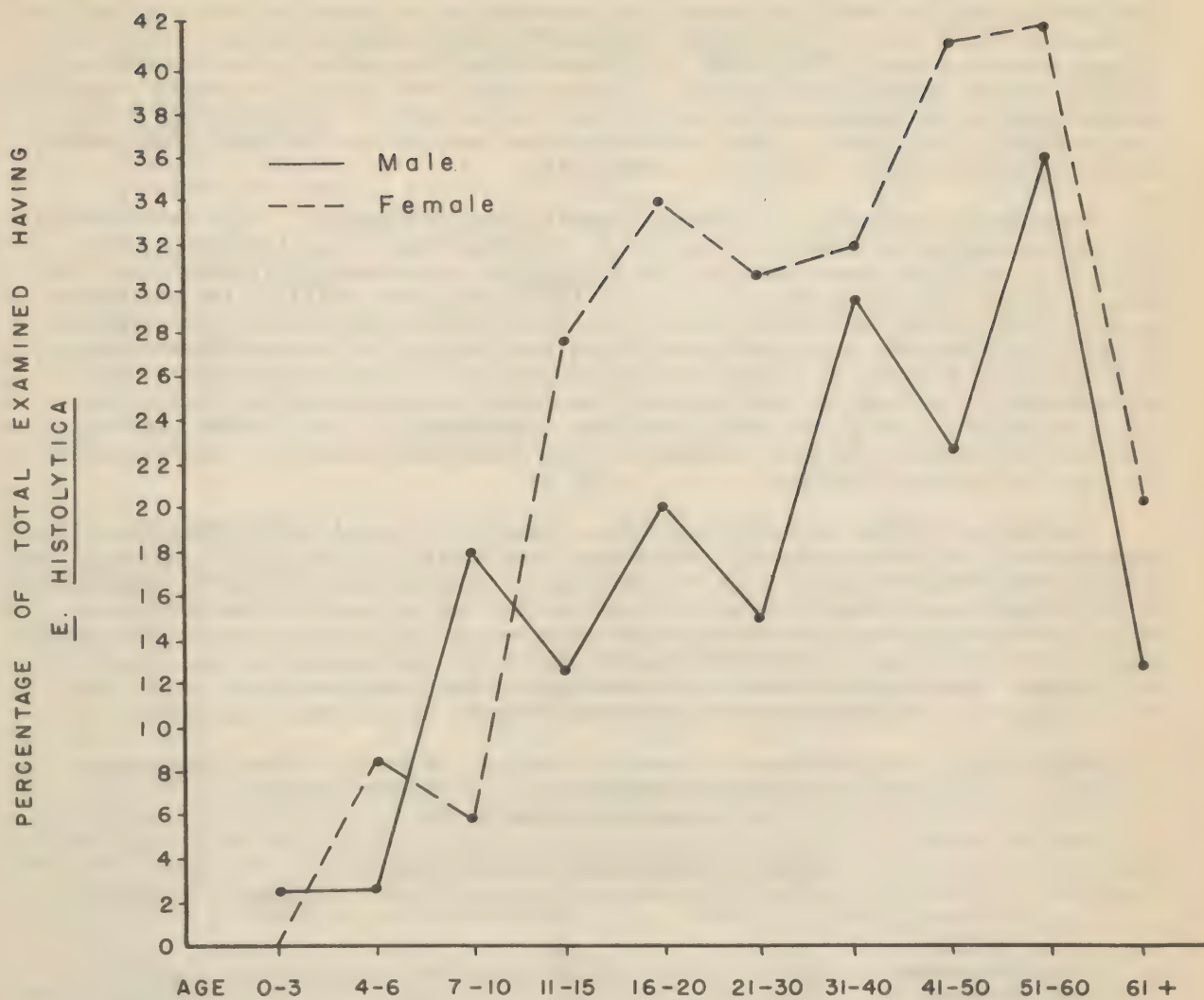


Figure 4. Infection with E. histolytica Based on Age of Males and Females

The occurrence of E. histolytica in families for which the primary food-handler was infected with this organism, was compared with families where a "non" food-handler only was so infected. A third category of comparison was introduced by selecting one individual from each family by chance, and determining the infection rate for families of those positive for E. histolytica. Supportive comparisons involving E. coli and E. nana were also made. The mother of the predominant generation of a household was selected as the primary food-handler. Although there were usually secondary ones, the person selected was recognized as the one most apt to reflect the influence of the food-handler. The husband of the food-handler was selected as the "non" food-handler; if not available another adult male was selected.

Considering families collectively for which the food-handler, "non" food-handler and chance-selected individual was positive, the incidence of E. histolytica, was 36.4, 35.1 and 38.3%, respectively. For E. coli the corresponding figures were 59.4, 61.1 and 65.0%; and for E. nana, 45.1, 48.6 and 48.9% (Table XVIII). The variations occurring here, with one exception, are not statistically significant; in the case of E. coli, the incidence was significantly less for families of infected food-handlers than for those of positive chance-selected individuals. These data indicate that transmission of protozoa by food-handlers was not a primary factor in establishing the high incidence among the population under consideration. It is quite possible that the influence of the food-handlers was not detectable because of superimposed infections due to other factors.

Considering the reciprocal groups, e.g., families of negative food-handlers, "non" food-handlers and chance-selected individuals (Table XIX), E. histolytica, E. coli, and E. nana were consistently lower in the families of negative food-handlers. Differences of statistical significance tended to occur between family groups segregated on the basis of food-handler and chance-selected individuals. Only in the case of E. histolytica was the difference between food-handler and "non" food-handler groups clearly significant. Deviations between "non" food-handler and chance-selected groups were not significant. An explanation for these differences has not been recognized.

Table XVIII. The Occurrence of Specific Protozoa in Family Groups Represented by an Infected Food-Handler, a "Non" Food-Handler Or a Chance-Selected Member

	<u>SELECTEES POSITIVE FOR E. HISTOLYTICA</u>			
	<u>FH</u>	<u>NFH</u>	<u>Chance</u>	<u>Total</u>
No. Families	64	42	32	138
Total Members	398	268	209	875
No. Infected	145	94	80	319
Percent Infected	36.4	35.1	38.3	36.4
Chi Square	0.001	0.221	0.298	0.519*
<u>E. COLI</u>				
No. Families	113	85	81	279
Total Members	678	540	509	1727
No. Infected	403	330	331	1064
Percent Infected	59.4	61.1	65.0	61.7
Chi Square	1.349	0.057	2.518	3.924*
<u>E. NANA</u>				
No. Families	94	62	58	214
Total Members	574	391	352	1317
No. Infected	259	190	172	621
Percent Infected	45.1	48.6	48.9	47.2
Chi Square	0.951	0.325	0.413	1.689*

* For Two Degrees of Freedom

P = 0.05 when chi square = 5.991

P = 0.01 when chi square = 9.210

Table XIX. The Occurrence of Specific Protozoa in Family Groups Represented by an Uninfected Food-Handler, "Non" Food-Handler, or a Chance-Selected Member

SELECTEES NEGATIVE FOR E. HISTOLYTICA

	<u>FH</u>	<u>NFH</u>	<u>Chance</u>	<u>Total</u>
Number Families	101	105	138	344
Total Members	607	654	826	2087
Number Infected	69	104	138	311
Percent Infected	11.4	15.9	16.7	14.9
Chi Square	5.978	0.516	2.122	8.616*

E. COLI

No. Families	52	62	89	203
Total Members	327	382	526	1235
No. Infected	95	124	182	401
Percent Infected	29.1	32.5	34.6	32.5
Chi Square	1.855	1.001	1.056	2.911*

E. NANA

No. Families	71	85	112	268
Total Members	432	533	683	1648
No. Infected	88	128	191	407
Percent Infected	20.4	24.0	28.0	24.8
Chi Square	4.348	0.133	3.922	8.403*

* For Two Degrees of Freedom

P = 0.05 when chi square = 5.991

P = 0.01 when chi square = 9.210

The incidence of infections for family groups of negative food-handlers, "non" food-handlers and chance-selected individuals was less than half that for families of positive selectees. This suggests the importance of familial association, which has been emphasized by previous investigators; however, it is believed that the nature of the tabulation introduced a bias in this regard. Other correlations will be made in an attempt to obtain a true evaluation of the effect of familial association.

Role of Mothers in Infecting Children - In order to determine what effect a mother might have on infecting her children with E. histolytica the pertinent data were analyzed (Table XX). Families were selected in which only the mother was infected, while all other members of her own generation (G-2) and her husband's parent's generation (G-1) were negative. For these observations, 34 families were selected among which there were 129 persons in G-3. Of the G-1 and G-2 only the mother (G-2) had E. histolytica. Of the 129 G-3 persons, 23 (18.6%) were infected. A similar group of 58 families were selected which contained 204 persons in G-3. In this case, neither the mother nor any other member of her generation (G-2) or her husband's parent's generation (G-1) were infected. Among the 204 G-3 persons, 21 (10.3%) were infected. In order to have a comparison for these findings, similar observations were made when only the father was infected. In this case, from 22 families in which the father was the only member of G-1 or G-2 infected, there were a total of 80 persons in G-3. Of these, 12 (15%) were infected. Information concerning the effect of an infected grandmother (G-1) on G-3, was too scanty to be of significant value. The following table summarizes the above findings.

It may be seen that there is only a slightly higher percentage of infection among children when the mother is infected than when the father is infected. This difference is not great enough to establish definitely any effect the mother may exert directly on infecting the children. When no adult in the family was infected the percentage of G-3 infection was understandably lower. This tends to corroborate the findings of other workers who have noted these familial characteristics of E. histolytica.

Table XX

	<u>No. Of Families</u>	<u>No. of Persons in Generation 3</u>	<u>No. and % Positive for <i>E. histolytica</i></u>
Mother with <i>E. histolytica</i> , all other G-1 and G-2 neg.	34	129	23 (18.6%)
No member of G-1 or G-2 infected	58	204	21 (10.3%)
Father with <i>E. histolytica</i> , all other G-1 and G-2 neg.	22	80	12 (15.0%)

A COMPARISON OF THE ZINC SULFATE AND THE FORMALIN-ETHER (406TH MGL) TECHNIQS:

Many technics have been devised to detect helminth ova and/or protozoan cysts. These may be classified according to features of manipulation and physical attributes as follows: (a) Direct smear (wet saline mounts, with or without stains, and permanently stained mounts); (b) sedimentation, followed by direct smear; (c) floatation procedures, with or without centrifugation; and (d) ether sedimentation with centrifugation. Another classification based on the range of effectiveness might be as follows: (a) Effectiveness limited to helminths; (b) effectiveness limited to protozoa; (c) effective for both helminth eggs and protozoan cysts; (d) effective for specific helminth eggs; and (e) adaptations for making egg counts.

At least in the minds of some, direct saline smears and permanent stains for protozoa are relegated to the identification of protozoan trophozoites and for confirmation of findings obtained by concentration methods.

The zinc sulfate flotation technic as developed by Faust et al (28) and Tobie et al (29) has been widely used and considered highly effective. It was the first concentration method which acceptably filled the dual role of recovering both eggs and cysts. Recently a formalin-ether sedimentation method (406th MGL technic) has also proven effective for the concentration of both eggs and cysts (23). This is a simplified form of the Telemann technic (30), with the addition of formalin-fixation as a means of reducing cyst distortion. A direct comparison of these two technics was undertaken by means of parallel examination on 161 single-stool specimens from a highly parasitized population.

Methods - In order to gain proficiency with the zinc sulfate technic, two technicians experienced in the detection and identification of both helminth eggs and protozoan cysts, prepared and examined a series of specimens by this method before the comparison was started. Critical attention was given to details of preparing the specimens; zinc sulfate with a specific gravity of 1.18 was used; and final recovery of eggs and cysts was by means of the wire loop. For any one group of specimens, the microscopic examination for both methods was done by the same technician.

Results and Discussion - (Tables XXI and XXII) With combined findings of both technics as 100%, the percentage recovered by each was used as the basis of comparison. Minimal advantages for the formalin-ether technic were noted for the recovery of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs. In the case of *Trichostrongylus orientalis* and *Schistosoma japonicum*, its efficiency exceeded considerably that of the zinc sulfate. (It should be noted that the 406th MGL method is mediocre for *S. japonicum* as compared with the AMS III method of Hunter et al) (31). The zinc sulfate appeared superior for the few cases of *Enterobius vermicularis* found, but the diagnosis of this parasite is dependent upon an anal-swab recovery of eggs.

For the helminths collectively, 92.4% of the total cases were diagnosed by the formalin-ether as compared to 80.2% by the zinc sulfate. The more effective diagnosis of *Ascaris* by the 406th MGL technic was quite definitely associated with the recognition

Table XXI. A Comparison of the Formalin-Ether (406th MGL) and Zinc Sulfate Technics

	Total Findings	Formalin-Ether No.	%	Zinc Sulfate No.	%
No. Persons Examined	161	161		161	
No. Parasitized	161	160		158	
No. with Helminths	161	159		158	
No. with Protozoa	109	107		87	
Helminths:					
<u>A. lumbricoides</u>	142	141	99.3	132	93.0
<u>T. trichiura</u>	149	143	96.0	128	85.9
Hookworm	101	93	92.1	87	86.1
<u>Trichostrongylus</u> sp.	45	33	73.3	18	40.0
<u>S. japonicum</u>	27	24	88.9	5	18.5
<u>E. vermicularis</u>	9	3	33.3	9	100.0
Total	473	437	92.4	379	80.1
Protozoa:					
<u>E. histolytica</u>	47	45*	95.7	21**	44.7
<u>E. coli</u>	84	81*	96.4	71***	84.5
<u>E. nana</u>	54	51*	94.4	32**	59.3
<u>I. butschlii</u>	4	4*	100.0	4***	100.0
<u>G. lamblia</u>	16	14	87.5	8	50.0
<u>C. mesnili</u>	2	1*	50.0	0	0
Total	207	196	94.7	136	65.7

* Essentially no distortion of cysts.

** Distortion excessive, making identification of the small race of E. histolytica particularly difficult

*** Some distortion, but identifications not greatly hampered

of unfertile eggs. Larger numbers of the fertile eggs were usually obtained by the zinc sulfate technic, but this advantage was offset by its relative ineffectiveness with unfertile eggs.

Observations on the relative efficiency of the technics for the detection and identification of protozoan cysts indicated an even greater advantage for the formalin-ether procedure. Of 47 cases of Endamoeba histolytica 45 (95.7%) were detected in contrast to 21 (44.7%) by the zinc sulfate method. This marked difference was clearly associated with the occurrence of the small race of E. histolytica (Table XXII). It has been shown by actual measurements that about 50% of the infections in the village from which the specimens were collected were of this type, e.g. measuring less than 10 microns in diameter. Of 21 cases so designated without actual measurements, only three were detected with the zinc sulfate technique, in contrast to 20 by the 406th MGL technic. The degree of difference was much less for infections in which the cysts measured 10 microns or over, the percentages being 64.7% and 94.1% respectively.

For 84 infections of E. coli, 96.4% were detected by the formalin-ether and 84.5% by the zinc sulfate technic. Correspondingly for Endolimax nana the figures were 94.4% and 59.3%, and for 16 cases of Giardia lamblia they were 87.5% and 50.0%.

In the case of E. histolytica and E. nana the amount of cyst distortion was the critical factor. Whereas the zinc sulfate causes considerable distortion, it is negligible with the formalin-ether procedure. With ether-sedimentation, precysts are not damaged. Distortion allows a personal factor to enter into the comparison, since the technician must evaluate what constitutes a diagnosable cyst. The nature of the

distortion produced by zinc sulfate is distinctive, and there is tendency, finally to identify on the basis of the distortion.

Table XXII. Relative Efficiencies of Technics for Small and Large Races of E. histolytica

	Total Cases	Formalin-Ether No.	%	Zinc Sulfate No.	%
Large Race	17	16	94.1	11	64.7
Small Race	21	20	95.2	3	14.3

An added advantage of the 406th MGL method is the fact that specimens may be preserved in formalin and kept for a period of time before concentration. This is convenient when the source of specimens is at a distance from the laboratory, or the rate of collection exceeds that at which the microscopic examinations can be done. Furthermore, the concentrated specimens do not require immediate examination, but may be held if the tubes are corked and placed in a refrigerator. The technic is known to be effective on helminth eggs other than those occurring in the comparison, including those of various trematodes and cestodes. Nematode larvae are also concentrated.

EFFICIENCY OF THE FORMALIN-ETHER CONCENTRATION TECHNIC: Repeated stool examinations (5 per person) performed in relation to an epidemiological study of intestinal protozoa afforded an opportunity to evaluate the actual efficiency of the formalin-ether (406th MGL) concentration technic (23).

Methods - The 10,216 specimens included in this study were examined as "unknowns". The technicians were unaware of the presence of any given parasite, thereby removing the possibility of undue searching for any particular species. Each of the subsequent four studies was made without knowing the findings of the previous examinations. A period of about 25 days was allotted for the work both in January and in September. It was accomplished under semi-field conditions on the railroad laboratory car. Each technician examined an average of 37 specimens per day. The results are presented in Tables XXIII (Protozoa) and Table XXIV (Helminths). The columns on the left indicate the new cases found by each of the five examinations, the center group of columns shows the total number of cases found up to, and including, the given examination, and the right hand group shows the cumulative percentages. For our purposes, the total number of parasites found by five examinations on the same person is taken as 100%, and used as the base line upon which the other values are determined.

An inspection of the values representing the cumulative percentages shows that these tend to follow the form of asymptotes. In order to find if this was true the method of X^2 was applied to the data obtained for each parasite. A basis of computing theoretical values for each parasite in each of the five successive examinations was obtained by using the average percent of cases revealed from the total of the five examinations. This value then represented the initial theoretical value and the subsequent four values were derived from it. After treating the data of each parasite in this manner it was found that in no case were the values of "P" less than 0.96, which indicated a valid assumption that both observed and theoretical values are commensurable.

The values of the mathematically calculated and actually-found efficiencies are plotted in Figures 5 and 6 and Table XXV. From these figures, one may tell the number of examinations required to attain any given percentage of the total, based on five examinations. Considering E. histolytica, 54% of the overall recognized cases were found on the first examination, while the calculated efficiency was 61%. Upon re-examination of the same persons, an additional 21% of the total was found. Likewise, 13, 9, and 3% of the total were found by the third, fourth, and fifth examinations.

Table XXIII. Efficiency of the Formalin-Ether Concentration Technic

- P R O T O Z O A -

Examination No.	<u>Total New Cases Per Exam.</u>					<u>Cumulative Cases</u>					<u>Cumulative % of all Cases Found After Each Examination</u>				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<u>E. histolytica</u>															
January	112	53	33	19	6	112	165	198	217	223	50	74	89	95	100
September	72	17	12	12	3	72	89	101	113	116	62	77	87	97	100
Total	184	70	45	31	9	184	254	299	330	339	54	75	88	97	100
<u>E. coli</u>															
January	391	66	18	25	17	391	457	475	500	517	76	88	92	97	100
September	268	46	25	14	8	268	314	339	353	361	74	87	94	98	100
Total	659	112	43	39	25	659	771	814	853	878	75	88	93	97	100
<u>E. nana</u>															
January	229	66	33	19	19	229	295	328	347	366	63	81	90	95	100
September	188	51	24	9	10	188	239	263	272	282	67	85	93	97	100
Total	417	117	57	28	29	417	534	591	619	648	64	82	91	96	100
<u>G. lamblia</u>															
January	58	26	15	17	17	58	84	99	116	133	44	63	75	87	100
September	81	33	18	15	9	81	114	132	147	156	52	73	85	94	100
Total	139	59	33	32	26	139	198	231	263	289	48	69	80	91	100

Table XXIV. Efficiency of the Formalin-Ether Concentration Technic

- H E L M I N T H S -

Examination No.	<u>Total New Cases Per Exam.</u>					<u>Cumulative Cases</u>					<u>Cumulative % of all Cases Found After Each Examination</u>				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<u>A. lumbricoides</u>															
January	748	48	15	16	13	748	796	811	827	840	89	95	97	99	100
September	727	56	26	17	16	727	783	809	826	842	86	93	96	98	100
Total	1475	104	41	33	29	1475	1579	1620	1653	1682	88	94	96	98	100
<u>T. trichiura</u>															
January	849	53	17	6	3	849	902	919	925	928	92	97	99	99	100
September	856	50	14	4	3	856	906	920	924	927	92	98	99	100	100
Total	1705	103	31	10	6	1705	1808	1839	1849	1855	92	98	99	100	100
<u>Hookworm</u>															
January	514	94	39	20	14	514	608	647	667	681	75	89	95	98	100
September	411	80	33	21	11	411	491	524	545	556	74	88	94	98	100
Total	925	174	72	41	25	925	1099	1171	1212	1237	75	89	95	98	100
<u>Trichostrongylus sp.</u>															
January	110	76	36	29	23	110	186	222	251	274	40	68	81	92	100
September	108	39	33	30	13	108	147	180	210	223	48	66	81	94	100
Total	218	115	69	59	36	218	333	402	461	497	44	67	81	93	100
<u>S. japonicum</u>															
January	139	95	52	26	27	139	234	286	312	339	41	69	84	92	100
September	143	63	41	36	18	143	206	247	283	301	48	68	82	94	100
Total	282	158	93	62	45	282	440	533	595	640	44	69	83	93	100

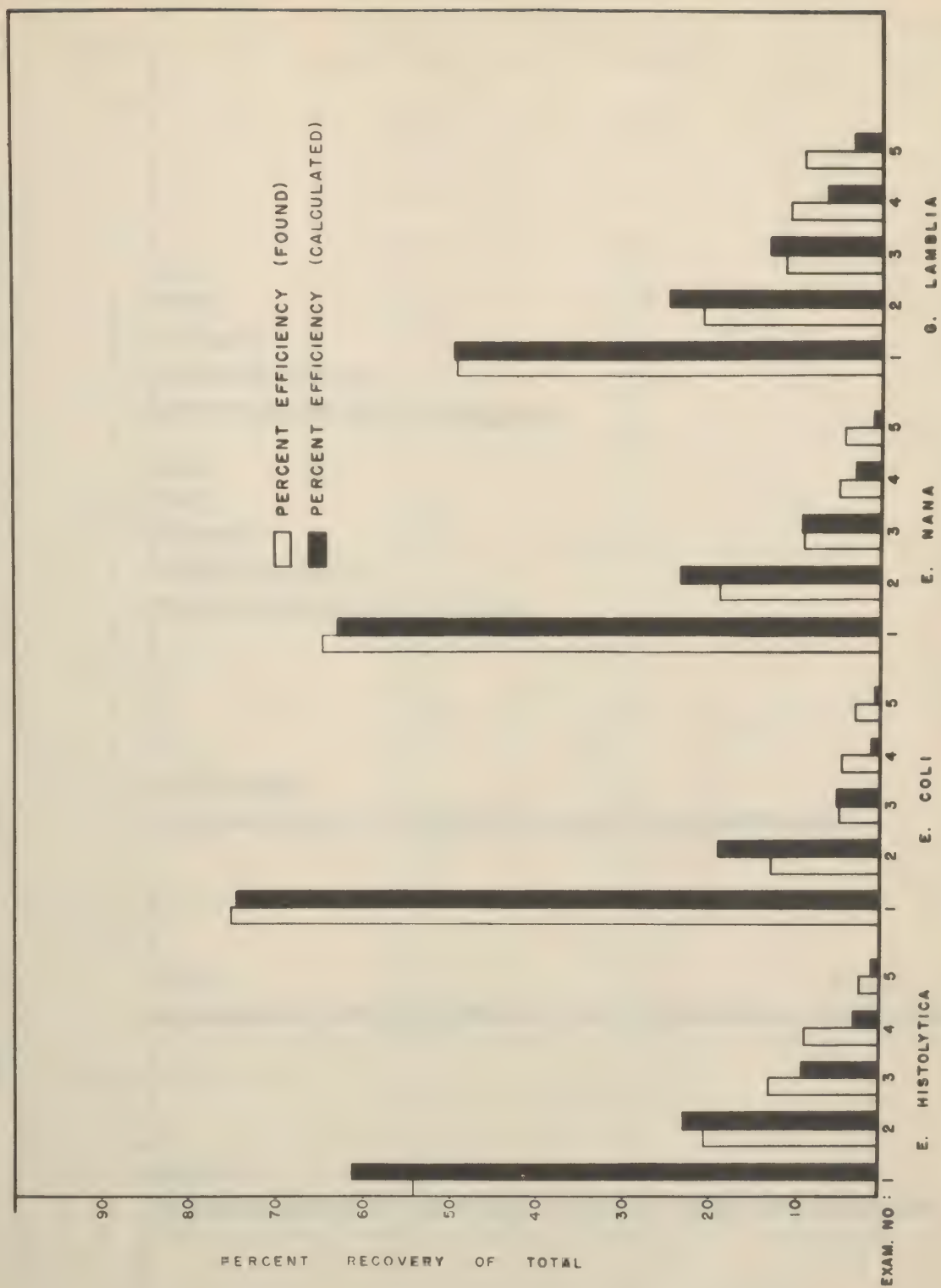


Figure 5. Percent Recovery (Based on the Total Number Positive by Species) of Protozoa by Successive Examinations of Steel Specimens with the Formalin-Ether Concentration Technic

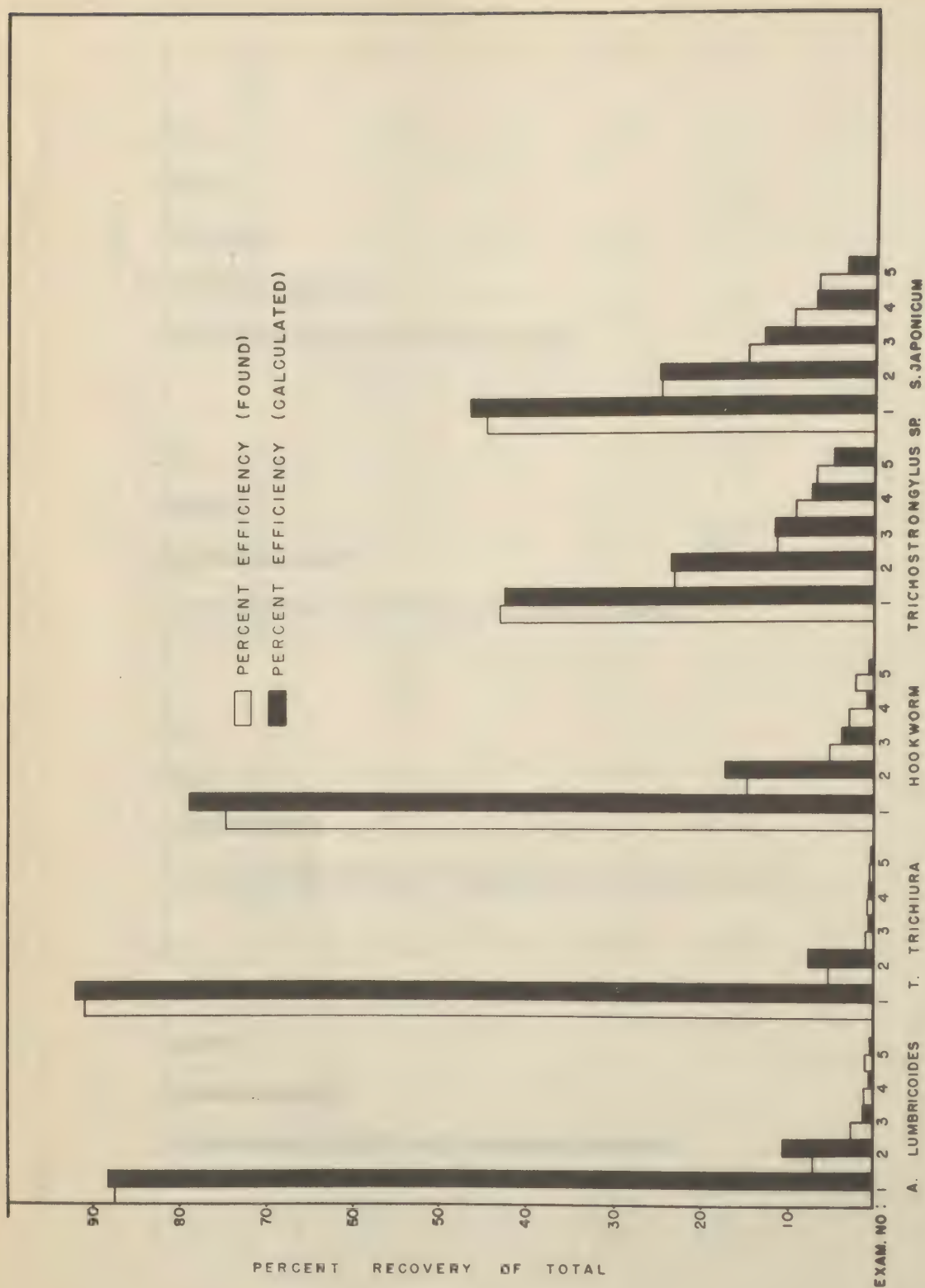


Figure 6. Percent Recovery (Based on the Total Number Positive by Species) of Helminth Ova by Successive Examinations of Stool Specimens with the Formalin-Ether Concentration Technic

Table XXV. Efficiency of the Formalin-Ether Technic

Calculated Efficiency Compared With Observed Efficiency

Parasite	% Efficiency (Observed)	% Efficiency (Calculated)	CHI ²	P**
<u>E. histolytica</u>	54.28	61.06*	0.5742	0.96
	74.93	84.83		
	88.20	94.09		
	97.34	97.69		
	100.00	99.10		
<u>E. coli</u>	75.05	74.80*	0.1970	0.99
	87.81	93.64		
	92.71	98.62		
	97.15	99.65		
	100.00	99.91		
<u>E. nana</u>	64.35	62.40*	0.0836	0.99
	82.40	85.73		
	91.20	94.63		
	95.52	97.98		
	100.00	99.24		
<u>G. lamblia</u>	48.09	48.23*	0.2316	0.99
	68.51	73.20		
	79.93	86.13		
	91.00	92.82		
	100.00	96.28		
<u>A. lumbricoides</u>	87.69	88.46*	0.0985	0.99
	93.87	98.67		
	96.31	99.84		
	98.27	99.98		
	100.00	99.99		
<u>T. trichiura</u>	91.91	92.26*	0.0321	0.99
	97.46	99.40		
	99.13	99.95		
	96.68	99.999		
	100.00	99.999		
Hookworm	74.77	77.31*	0.1679	0.99
	88.84	94.85		
	94.66	98.83		
	97.98	99.73		
	100.00	99.94		
<u>Trichostrongylus sp.</u>	43.86	42.61*	0.1404	0.99
	67.00	67.06		
	80.88	81.43		
	92.75	89.34		
	100.00	93.88		
<u>S. japonicum</u>	44.06	46.78*	0.1274	0.99
	68.75	71.67		
	83.28	84.92		
	92.96	91.97		
	100.00	95.73		

* This value is the average percent of efficiency as determined from the 5 consecutive trials

** Calculated from Fisher's "Statistical Methods for Research Workers"

Seventy-five percent of the *E. coli* were recognized on initial examination while the subsequent examinations revealed 13, 5, 4, and 3% new cases. In the case of *Ascaris*, 88% of the cases were immediately diagnosed while the second examination revealed only 6% new cases. The remaining three examinations each gave 2% new cases. In similar manner, 92% of all the whipworm cases were recognized by a single study with the four subsequent examinations revealing only 6, 2, 1, and 0.03% new cases. By considering a single examination, the general value of subsequently expected values may be determined by referring to these figures.

MASS TREATMENT OF ENDAMOEBIA HISTOLYTICA CARRIERS: It is generally believed that approximately 10% of the Japanese harbor *Endamoeba histolytica*. Symptoms are not generally manifested in a preponderance of the infections, and some question has been raised as to the value of treating such asymptomatic individuals (32). Nevertheless, most investigators feel that such treatment should be administered, not only to cure the asymptomatic patient, but to lower the incidence of the disease.

Materials and Methods - From a series of families in the villages of Mutsu-awa and Tatomi, Yamanashi Prefecture, Japan, 241 individuals harboring protozoa were chosen for treatment. Diagnoses were based on 5 fecal examinations per individual using the formalin-ether concentration technic. Each person was interviewed prior to treatment and their symptoms, if any, were noted. Those who were senile, or under the care of a physician, were excluded from this study. Those who had albumin and/or urobilinogen in the urine were likewise excluded. The drugs in the dosage given below were administered over a period of two weeks, Carbarsone for the first week, and Chiniofon for the second.

Age Group	Carbarsone - Daily	Chiniofon - Daily
3 - 6	0.25 gm X 1	0.25 gm X 3
7 - 11	0.25 gm X 2	0.50 gm X 3
12+	0.25 gm X 3	0.75 gm X 3

Each 3 days the individuals were interviewed and their symptoms and drug side-reactions, if any, noted. Those who for some reason had not taken the drug were eliminated from the study. Five weeks, and again 7 months, after treatment, each patient had a series of 5 post-treatment examinations. Of 124 patients harboring *E. histolytica*, 18 (15%) were excluded for various reasons. Of the remaining 106, only one was demonstrated to harbor the organism 5 weeks after treatment. Seven months after treatment, 4, or 3.8%, were found to harbor *E. histolytica* (Table XXVI). This treatment-failure rate is indicative of a high level of therapy efficiency. It is possible that because of the small stature of the Japanese the dosage of drugs may have been comparatively large. However, comparable results were reported with Carbarsone and Diodoquin for persons infected during the Mantetsu epidemic (33).

Table XXVI. Effectiveness of Treatment for Intestinal Protozoa With Carbarsone and Chiniofon

	No. Treated February, 1952	Treatment Failures		
		March, 1952*	September, 1952	
		No.	No.	%
<i>E. histolytica</i>	106	1	4	3.8
<i>E. coli</i>	162	9	18	11.1
<i>E. nana</i>	128	25	33	25.8
<i>I. bütschlii</i>	24	0	0	0.0
<i>G. lamblia</i>	31	15	19	61.3

* There was an interval of about 5 weeks between the end of the treatment and the first post-treatment examination

An evaluation of results in relation to the benefit of mass treatment can best be done by comparing the findings in September with the pre-treatment findings. For Mutsusawa village the incidence of E. histolytica was 28.8% in January and 4.1% in September. The latter figure includes treatment failures, inadequate dosages, and reinfections. For Tatomi the corresponding figures were 13.5 and 2.2%. Ultimate benefits from mass treatment will depend on how quickly reinfections occur.

SKIN-TESTS FOR AMEBIASIS: A small group of persons with amebiasis were skin-tested with an antigen of Endamoeba histolytica. Five laboratory personnel were initially tested as controls. Four of these gave no detectable reaction, while the fifth (probably a false positive) gave a typical immunologic wheal which subsided normally without further reaction. Twenty-nine individuals with amebiasis, as proven by stool examination, were then tested with antigen dilutions of 1:8, 1:16, and 1:64. Procedures and the reading of reactions were on the same basis as skin-tests performed for schistosomiasis and paragonimiasis (34).

With 1:8 and/or 1:16 antigen dilutions, wheals, similar to those for schistosomiasis and paragonimiasis, were noted in 21 persons, or 72.4% (Table XXVII).

A comparison of the several antigen dilutions shows that the 1:64 dilution was beyond the limits of effectiveness, giving only one-third as many positives as the 1:8. The advantage of the 1:8 dilution over the 1:16 was minimal (18 and 17 positives, respectively).

The possibility of the doubtfuls being evaluated as positives is entertained. The wheal size in general was smaller as compared with those obtained with Schistosoma japonicum and Paragonimus antigens. The 2 mm. wheals were well defined reactions, and with one exception, the associated controls gave no reaction. The evaluation of these smaller wheals must await testing of larger numbers of known negatives.

Table XXVII. Reactions of Persons with Amebiasis to Intradermal Injections of an Amebic Antigen

Increase of Wheal Over Controls	Antigen Dilutions			Controls**
	1:8	1:16	1:64	
0-1 mm (Negative)	3*	5*	17*	24
2 mm (Doubtful)	8	7	6	5
3-4 mm (Positive ***)	14	11	5	1*
5-6 mm (Positive ***)	2	3	1	
7+ mm (Positive ***)	2	3		
Total Positives	18	17	6	
Total Cases	29	29	29	
Percent Positive	62.1	58.6	20.7	

* For one person the control as well as antigen gave positive reaction

** Merthiolated saline

*** Using both 1:8 and 1:16, 21 patients were positive

IMMUNOLOGIC INVESTIGATIONS ON ASCARIS - The Antigenicity of Various Body Parts of Ascaris suum - Serologic studies of parasitic infections have focused considerable attention on the chemical nature of the antigenic agent, but only minimal consideration has been given to its origin in the body of the parasite. There is some reason to believe that certain parts of a worm are more actively and specifically antigenic than others. Investigations concerning this matter have been initiated, using Ascaris suum which is abundant and lends itself readily to separation of body parts.

Saline extracts of whole worm, cuticle, and internal organs (including the coelomic fluid) were prepared and approximately a 1:10,000 antigen dilution injected intradermally. Since phenol was used as a preservative, a phenol saline control was used.

The results of the tests are shown in Table XXVIII. Whole worm extracts showed marked antigenic activity. There was a wheal increase of 3 mm. or more in 39 of 41 positive persons. The two cases showing increases of 2 mm. were designated as doubtful. All controls were negative. In contrast, the extracts of cuticle and internal organs produced no reaction in 37 and 39, respectively, of the 41 cases. Whereas the reliability of the whole worm extract was 95%, that of the two specific body parts was less than 5%. It appears then, that the antigenic agent is localized in either the epithelial or muscular layer of the body wall. It is proposed to attempt extractions of these layers.

Table XXVIII. The Antigenicity of Various Body Parts of Ascaris suum
As Determined by Intradermal Tests on Human Cases

Increase of Wheal Over Controls	Whole Worm	Cuticle	Internal Organs	Controls
0-1 mm (Negative)	0	37	39	41
2 mm (Doubtful)	2	2	1	0
3-4 (Positive)	18	2	1	0
5-6 (Positive)	16	0	0	0
7+ (Positive)	5	0	0	0
Total Positives	39	2	1	0
Total Cases	41	41	41	41
Reliability	95.1%	4.9%	2.4%	0.0%

Duration of Sensitivity to Ascaris Antigen Following Complete Elimination of Worms - An opportunity became available to determine the length of time that the skin-test for ascariasis remains positive after the infection is eliminated. Dr. Nagano of the Kitasato Institute had been attempting by means of repeated treatment to eliminate ascaris from the isolated island of Hatsushima located off the coast of Shizuoka Prefecture, Japan. Examinations and treatment had been carried out monthly for fifteen months. Some people were originally negative and others became negative intermittently throughout this period. At the time of the tests about 25 light infections remained among a population of approximately 280. Of this group, 216 were skin-tested with the whole-worm ascaris antigen. The figures in Table XXIX indicate that no reduction in sensitivity had occurred even for those who had been free of infection for more than 15 months. Skin sensitivity, therefore, may be retained for well over one year. It may prove appropriate to repeat the tests at Hatsushima at a later time. One person, hypersensitized 20 years previously by handling ascarids for research purposes, and who with fair certainty had not been infected in the interim, gave an extreme reaction, exceeding anything observed among 250 infected persons. The injection site increased from 3 to 30 mm in 10 minutes followed in 24 hours by dermal edema of about 60 mm in diameter.

A correlation of reaction with age indicated that all ages except the 1-5 year old group reacted equally to the skin-test antigen; of 17 of the youngest group, only 7 were positive.

As a possible group of negative controls, 59 American personnel were skin-tested. Approximately 20% gave positive reactions. Of about half this group who submitted stool specimens, only one was found to harbor ascaris. This raises the question regarding false positive reactions. Another explanation would be that the test reflects the infections people may have previously had during their life. The observations at Hatsushima suggest the latter. A conclusive answer to this question will require a group of individuals as negative controls who with certainty have not been exposed

to ascaris. It would appear that skin-tests as a diagnostic procedure for ascariasis, even in an area of low endemicity, would not be practical.

Table XXIX. Duration of Sensitivity for Ascaris Antigen

	<u>Free Of</u> <u>Ascaris For</u>	<u>Number</u> <u>People</u>	<u>Skin-Test</u> <u>Positives</u>	<u>Percent</u> <u>Positive</u>
I	15 months or over	33	30	90.9
II	10 - 14 months	41	34	82.9
III	6 - 9 months	36	32	88.9
IV	1 - 5 months	97	87	89.7

SEASONAL STUDIES ON ASCARIS LUMBRICOIDES: A previous study involving observations on seasonal fluctuations of incidence and intensity of Ascaris lumbricoides infections has been reported (4). Beginning in February 1952, and continuing to the present time, stools were collected periodically at intervals of four to six weeks from a group of fifty families (291 individuals) living in the hillside villages of Mutsusawa, Yamanashi Prefecture, Japan. These families were thought to represent a cross-section of the population in this area. Eight specimens have thus far been collected from each person.

Egg counts of ascaris, whipworm, and hookworm were made on each specimen by the following method: (a) The stool was thoroughly comminuted and strained through two layers of gauze into a graduated centrifuge tube; (b) the tube was then centrifuged for five minutes at 2500 rpm; (c) the supernatant liquid was poured off, the volume of the sediment measured and formalin (an amount equal to twice the volume of sediment) was added; (d) one drop of this material was placed on a slide with a graduated 0.6 ml pipette and covered with a square, 22 mm cover slip; (e) five of fifteen low power lanes (Nos. 1, 4, 7, 10, and 13) of this preparation were counted and this total was multiplied by 180 to determine the number of eggs per ml. of strained, centrifuged feces. Although the soundness of this egg-counting procedure has not as yet been proved, it was carefully standardized to assure valid comparisons.

As will be seen from Figure 7, there are apparently two peaks of infection; the highest peak occurred in July, with a lesser one in April. There was a definite decrease from April to May. From the peak in July there is a slight decrease in August, after which there is a fairly steady decline to the lowest point of infection in December. Upon comparison of the median egg count with the average count, it can be seen that the curve follows essentially the same pattern except for a very slight deviation from July to August. The median egg count by month is, however, considerably lower, with an average difference of 1581 eggs.

The incidence of ascaris infections was relatively uniform for all collections from February through August, with deviations of only 3.0% from the peak of 85.0 in April and May. For September and October the figure was about 76.0%, while in December there was a further decline to about 66.0%.

It is planned to continue the project through May, 1953 to determine, if possible, whether there is actually a decline in egg production during the spring.

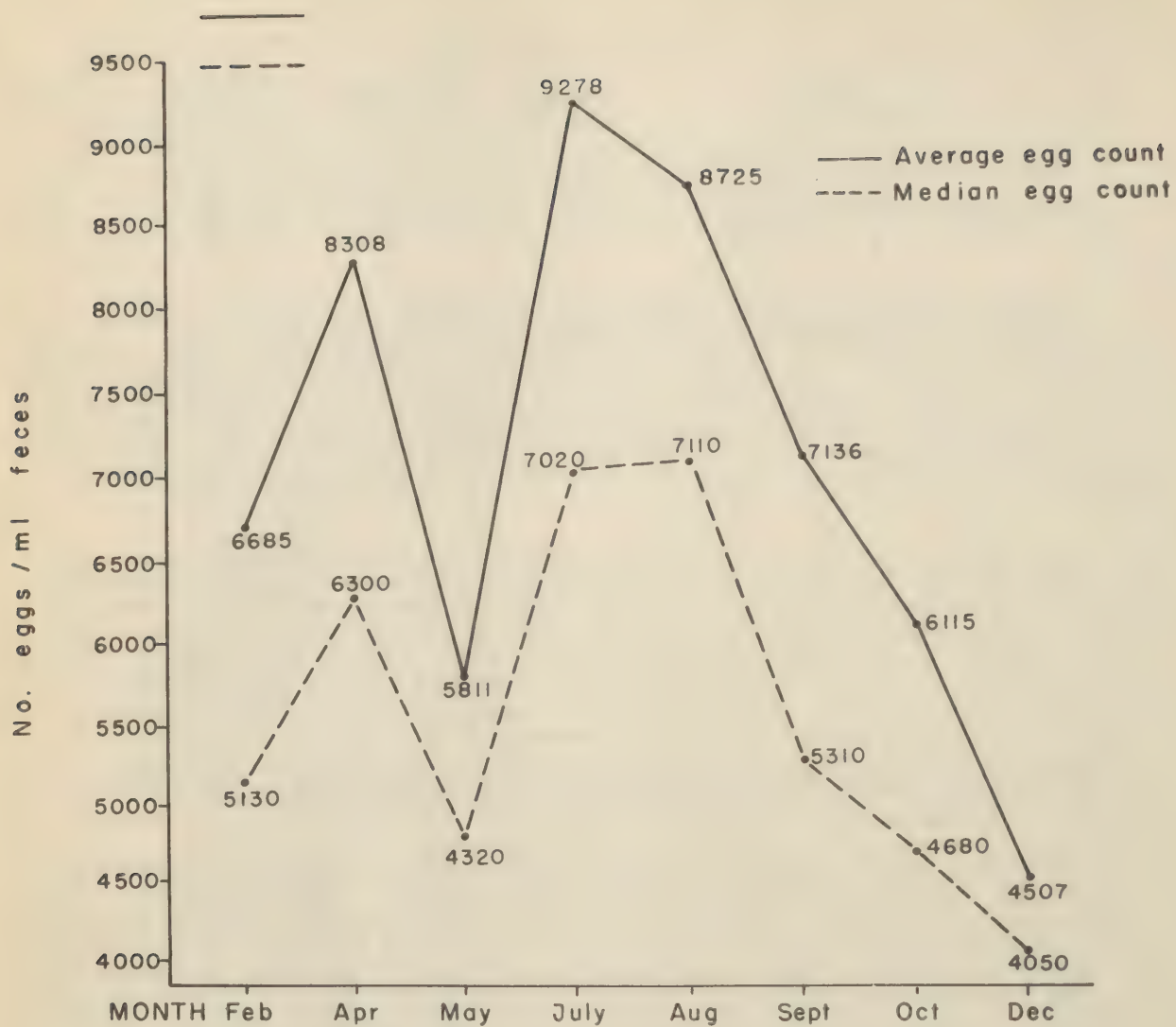


Figure 7. Average and Median Egg Counts for *A. lumbricoides*

DEPARTMENT OF SEROLOGY

Exclusive of investigative work, 264,354 procedures were completed in 1952. Details are summarized in Table I.

Table I. Routine Tests

General:

Cardiolipin Microflocculation (Qualitative)	73,816
Cardiolipin Microflocculation (Quantitative)	10,387
Cardiolipin Complement Fixation (Serum & Spinal Fluid ...	14,109
Pandy	2,277
Colloidal Gold	2,279
Heterophile Agglutination	1,124
Cold Agglutination, Donath-Landsteiner, Misc.	121
Total	104,113

Immunohematology:

Blood Grouping & Subgrouping (Direct & Indirect)	2,064
Rh ₀ Determination (Homozygous; Heterozygous)	928
Rh Antigen Analysis (Phenotypic; Genotypic)	58
Rh Antibody Detection (Enzyme Tests Included)	1,043
Rh Antibody Titration (Enzyme Tests Included)	132
Coombs Test (Direct; Indirect; Titrations)	194
M, N, Duffy Kell, D ^u , Miscellaneous	46
Total	4,465

Blood Processing (Blood Bank)*

Group "O"	15,983
Group "A"	13,535
Group "B"	3,869
Group "AB"	1,367
Units Processed	34,754
Total Processing Procedures	155,776

GRAND TOTAL **264,354**

* Group and Rh percentages are not presented because the figures shown include multiple donations from individual donors. Such replicate data would invalidate the frequencies computed.

CARDIOLIPIN MICROFLOCCULATION ANTIGEN: Discrepant results were observed with two different lots of stock cardiolipin microflocculation antigen. One was presumably older than the other, dates of preparation not having been recorded. Otherwise, their distributor considered them similar in all respects.

A systematic inquiry into the observed discrepancies was made using 972 sera of different reactive categories. Results are shown in Table II. Since all antigen lots are stored and used under identical conditions, the results imply 1) that separate lots vary in reactivity to begin with, or 2) that their reactivities, initially uniform, undergo different rates of change with storage.

Table II. Cardiolipin Microflocculation Reactions
With Old and New Antigens

No. of Sera Tested	Antigen Lots	
	Old	New*
182	Disagreement	
109	Rough	Neg.
31	1/	Neg.
4	1/	Neg.
5	1/	Rough
6	1/	1/
3	2/	Neg.
4	2/	1/
4	2/	1/
1	3/	Rough
5	3/	1/
2	3/	2/
1	4/	1/
5	4/	3/
1	1/	2/
1	Neg.	Rough

790

Agreement in All Categories

* Agreement between cardiolipin microfloc-
culation and cardiolipin complement fixation
results was much better with the new than with
the old lot.

CARDIOLIPIN COMPLEMENT FIXATION ANTIGEN: In an attempt to improve STS, a given antigen (procured as a standardized reagent) was titrated to establish the concentration optimal for the Wassermann test. The titration, summarized in Table III, shows two optima. The first, 1:130, is based on the largest amount of antigen giving a 4/ reaction with the smallest amount of antiserum. The second, 1:163, represents the smallest amount of antigen giving a 4/ reaction with the smallest amount of antiserum.

Table III. Titration of Cardiolipin Antigen Used In The
Complement Fixation Test for Syphilis (STS)

Serum Dilution	1:65	1:98	1:130	1:163	1:245	1:368
1:100	Neg.	Neg.	Trace	Neg.	Neg.	Neg.
1:40	1/	1/	4/	4/	3/	2/
1:20	4/	4/	4/	4/	4/	4/
1:10	4/	4/	4/	4/	4/	4/
1:5	4/	4/	4/	4/	4/	4/

To determine whether the two optimal dilutions would give rise to different results under the same test conditions, parallel Wassermann tests were done. Reactions with 40 sera and 5 spinal fluids are compared in Table IV. They indicate good agreement regardless of the antigen dilutions employed.

It may be assumed that essential accord was attained because the two dilutions were situated within limits of the optimal zone. Had one or the other departed from these limits, it is conceivable that greater disagreement would have resulted.

Table IV. Comparison of Wassermann Test Results Using Two Optimal Dilutions of Antigen 1:130 and 1:163

Serum No.	1:130	1:163	Serum No.	1:130	1:163
4299	Neg.	Neg.	4872*	4	1
4309	Neg.	Neg.	W 215	2	2
4296	Neg.	Neg.	W 239	2	2
4598	Neg.	Neg.	W 222	3	3
4735*	Neg.	Neg.	W 313	3	3
5267	Neg.	Neg.	W 326	3	3
5323	Neg.	Neg.	5543	3	3
5338	Neg.	Neg.			
5415	Neg.	Neg.	W 304	4	3
5639	Neg.	Neg.	4321	4	4
5771	Neg.	Neg.	4606	4	4
5791	Neg.	Neg.	4612	4	4
W 217	Neg.	Neg.	4613*	4	4
W 227	Neg.	Neg.	4634	4	4
W 234	Neg.	Neg.	4635	4	4
W 295	Neg.	Neg.	4675	4	4
W 320	Neg.	Neg.	4723	4	4
W 321	Neg.	Neg.	5358	4	4
W 323	Neg.	Neg.	W 211	4	4
			W 214	4	4
4709*	Trace	4	W 242	4	4
4905*	Trace	Trace	W 314	4	4
5308	Trace	Trace	W 327	4	4
W 324	Trace	Trace			

* Spinal Fluid

EFFECT OF INACTIVATING SERA FOR CARDIOLIPIN WASSERMANN TESTS: A study was undertaken to determine whether human complement was restored in sera inactivated but not tested for several hours. Normal and luetic sera containing varying amounts of reagin were inactivated at 56°C for 30 minutes. Three to five hours later each serum was divided into two portions, only one of which was reactivated at 56°C for 10 minutes. Both aliquots were then Wassermann-tested in the conventional manner.

An additional experiment was included to examine the effect of inactivation on two full units of guinea pig complement. For this purpose the usual serum anticomplementary control system (antigen excluded) was used. Following overnight refrigeration one set of controls was inactivated at 56°C for 10 minutes; another was inactivated at 56°C for 20 minutes; the third remained uninactivated. All controls were then mixed with sensitized red cells, incubated and read.

Results are summarized in Table V. The essential similarity of reactions in sections (a) and (b) suggest that little if any complementary factors reformed 3 to 5 hours after initial inactivation. However, it has been observed elsewhere that more prolonged storage of inactivated serum may tend to restore complement activity. In such instances reactivation is recommended.

Section (c) reveals that 1) omission of heating permitted full hemolytic activity of two units of guinea pig complement (negative reaction); 2) heating for only 10 minutes at 56 C partially or completely inactivated the two units; 3) heating for 20 minutes consistently and completely inactivated the two units (4/ reaction).

SERODIAGNOSIS OF PAROXYSMAL HEMOGLOBINURIA: Two cases of suspected paroxysmal hemoglobinuria were referred for serologic study.

Table V. Effect of Inactivation on Wassermann Reactions

(a) 148 Test Sera Not Reactivated*		(b) 148 Test Sera Reactivated**		(c) 296 Anticomplementary Control Systems***	
Neg.	66	Neg.	66	Neg.	148 (Uninactivated)
Trace	7	Trace	6	Trace	0 (Inactivated 10 min.)
1/	4	1/	5	1/	1 (Inactivated 10 min.)
1/	4	1/	5	1/	0 (Inactivated 10 min.)
2/	4	2/	3	2/	7 (Inactivated 10 min.)
3/	4	3/	3	3/	23 (Inactivated 10 min.)
4/	59	4/	60	4/	13 (Inactivated 10 min.)

* Sera inactivated initially at 56°C for 30 minutes; 3 to 5 hours later incorporated into immune system without reactivation.

** Sera inactivated initially at 56°C for 30 minutes; 3 to 5 hours later reactivated at 56°C for 10 minutes and incorporated into immune system.

*** 148 controls uninactivated, 44 inactivated at 56°C for 10 minutes and 104 for 20 minutes.

Case I, a 24 year old white soldier from Colorado, claimed that as a child his urine occasionally seemed to show red discoloration. He first definitely observed bloody urines at the age of 15 after playing in the snow. This condition occurred about 4 times a year. Hemoglobinuria reappeared while he was serving in Korea, again in Japan following a cold shower, and currently after a chilly ride from his hospital to the 406th Medical General Laboratory, where his blood was drawn for the tests to be described. He denied venereal disease; however, an evacuation hospital records him to be serologically positive. He asserted that he did not receive antiluetic treatment.

Case II, a 22 year old white soldier from Missouri noticed hemoglobinuria at the age of 10. Later he connected this condition and its attendant discomfort and back pain with exposure to cold. In 1950 he had a positive serology. He admitted frequent exposures but disclaimed having venereal lesions. After subsequent antiluetic treatment his serology reverted to negative. Currently his hospital laboratory reports him positive again. He has Hutchinson's teeth and says his father also had Hutchinson's teeth.

Cold agglutinations, STS, and Donath-Landsteiner tests were then performed. Specimens from Patients I and II failed to demonstrate cold agglutinins but were 4/ by Wassermann and microfloculation tests, with quantitative titers of 1:8 and 1:32 respectively.

Bloods for the Donath-Landsteiner test were drawn with warm equipment and processed into serum and cell suspension components while warm. Bloods from normal persons of identical ABO groups as the patients were treated similarly and served as controls. The Donath-Landsteiner reactions were positive for both patients as shown by hemolysis in tubes 1 and 3 of the protocol given in Table VI. These findings, with the STS results and the clinical data, are diagnostic of paroxysmal hemoglobinuria.

PREPARATION OF RARE ANTISERA AGAINST HUMAN ERYTHROCYTE ANTIGENS: Attempts were made to prepare anti O, anti Hr₀ (anti d) and other immune sera. As a start, rabbits 4R and 4V were injected intravenously with a total of 0.875 cc each of packed, washed red cells. 4R received O, M, CcDEe erythrocytes twice weekly for 2 consecutive weeks and 4V received O, N, ccdde cells under an identical schedule. Five days after the last injection each animal was trial-bled and its serum examined for antibodies. The results of agglutination tests with unabsorbed and absorbed sera appear in Table VII.

Table VI. Donath-Landsteiner Test

Reagents		1	2	3	4	5	6
Patient's serum	cc	0.25	-	0.25	-	-	-
Patient's rbc, 5%	cc	0.1	-	-	0.1	0.1	-
Control Serum	cc	-	0.25	-	0.25	-	-
Control rbc, 5%	cc	-	0.1	0.1	-	-	0.1
G pig Complement, 1:10	cc	0.1	0.1	0.1	0.1	0.1	0.1
Saline, 0.85%	cc	0.05	0.05	0.05	0.05	0.03	0.03

Immerse rack in ice water for 10 min., incubate in 37°C water bath for 30 min. and read. A duplicate rack which is not chilled constitutes the control.

RESULTS

Patient I:	Test	H*	N**	H	N	N	N
	Control	N	N	N	N	N	N
Patient II:	Test	H	N	H	N	N	N
	Control	N	N	N	N	N	N

* Hemolysis

** No hemolysis

It is apparent from the table that both animals were successfully immunized against human erythrocytes as a whole. To establish the presence of definitive anti O hemagglutinins, it was necessary to absorb the respective antisera with cells having antigens common, except for O, to those used in the immunizations. As recorded, the two absorbed sera agglutinated O, M, CcDEe and O, N, ccddee cells in dilutions of 1:80 and 1:40 respectively, thus indicating the presence of O antibodies. The weak agglutination noted with A₁B, MN, CcDEe erythrocytes, the only reasonably appropriate cells available, probably was due to residual antibodies. These were not completely removed because absorptions were discontinued when the antiserum began to show marked hemolysis. Absorptions with the A₁B,N,ccddee erythrocytes, however, went to completion.

To verify the preliminary findings, absorbed 4V serum was tested with 10 group O, 2 group A₂, 4 group A₁, 2 group B, 1 group A₂B and 2 group A₁B bloods. Agglutination was observed only with the O and A₂ bloods, the latter possibly being A₂O.

Additional experiments were then carried out to demonstrate the possible presence of anti Hr₀ (anti d). The 4V antiserum was first absorbed with O,M,CcDEe cells (Japanese source), thus removing O and e agglutinins. It was further absorbed with A, MN ccdDee cells (Negroid source) to remove the N and c agglutinins. Because, statistically, both kinds of cells are thought to be high in Rho.Rho (D/D) frequency, it was hoped that all but anti d agglutinin would be absorbed, hence leaving it exclusively to react with its homologous Hr₀ (d) antigen.

Accordingly, serum so absorbed was tested with rh-negative cells which had been treated with papain to enhance reaction sensitivity. No agglutination was observed. If the absorbing cells were truly homozygous for Rh₀ (D) and hence contained no Hr₀ (d), this negative finding supports the opinion that the postulated Hr₀ (d) factor is poorly antigenic.

To assay the 4V antiserum for anti N agglutinins, extraneous antibodies were removed by absorbing with O, M, ccddee cells. The absorbed serum was then tested with 18 varieties of cells. All erythrocytes known to contain N or MN antigens were agglutinated, whereas all with M antigen were not.

Table VII. Anti O Hemagglutinins in Sera of Immunized Rabbits

Antiserum			Antiserum Dilutions											
Prepared Against	Absorbed With	Tested With	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	1:20480
rbc	rbc	rbc												
	None	O,M,CcDEe	R*	1/	3/	4/	4/	4/	4/	4/	4/	2/	1/	-
O,M,CcDEe (4R)		O,M,CcDEe	4/	4/	2/	1/	-	-	-	-	-	-	-	-
	A ₁ B,MN,CcDEe	A ₁ B,MN,CcDEe	1/	1/	-	-	-	-	-	-	-	-	-	-
	None	O,N,ccdde	R	1/	2/	3/	4/	4/	4/	4/	4/	4/	R	-
O,N,ccdde (4V)		O,N,ccdde	2/	2/	1/	R	-	-	-	-	-	-	-	-
	A ₁ B,N,ccdde	A ₁ B,N,ccdde	-	-	-	-	-	-	-	-	-	-	-	-
* Rough														

* Rough

COLD-WARM AGGLUTININS IN PREGNANCY: Thirteen blood specimens from an rh-negative (O,N,ccddee) patient receiving prenatal care were collected at frequent intervals and tested for Rh antibodies. None of these samples revealed hemagglutinins. Aypically, a fourteenth obtained just before term gave positive reactions with Rh-positive (CDE) and Rh-negative (cde) erythrocytes, suggesting the possible presence of both Rh and Hr antibodies. Since coexistence of both kinds of antibodies in a completely rh-negative homozygous individual (ccddee) is extremely improbable, attempts to identify the observed antibodies pursued different lines of approach.

Heterophile antibodies were excluded when no agglutination was demonstrated with sheep erythrocytes. Hemagglutination of human red cells in albumin but not in saline systems (enzyme-treated cells excepted) suggested the presence of occult antibodies. However, this possibility was eliminated after direct and indirect Coombs tests failed to show positive reactions. Examination for autohemagglutinins revealed that in a high protein system the patient's serum reacted with her own cells at 4° but not at 37° and 57°C. These findings pointed to further search for thermagglutinins. Results of such studies are summarized in Table VIII. The data tabulated show that a cold-warm variety of homologous hemagglutinin was detected in the patient's blood just before parturition. These antibodies were demonstrable at 4°, 25° and 37°C but not at 56°C. Further, they could be detected only in high protein systems. An exception was observed when enzyme-treated erythrocytes in saline suspensions were also agglutinated. In the latter case, however, warm agglutinins were manifested at 25°C only and in low titer.

Of clinical interest is the observation that despite a three day delay in parturition and despite the presence of cold-warm agglutinins, pregnancy terminated without incident. Two days postpartum the patient's serum gave similar results as the preparum specimen.

Rh AND ABO BLOOD GROUP DISTRIBUTIONS IN JAPANESE: 4,541 Japanese, representing a realistic cross section of the indigenous population, were examined to determine their Rh and ABO group distributions. The ABO groups were distributed as follows: O, 33.1%; A, 36.5%; B, 21.6%; AB, 8.8%. Compared to the observations made by other investigators these percentages are moderately higher for group O and slightly lower for groups A, B, and AB.

In the present study 4,521 (99.56%) Japanese were Rh-positive and 20 (0.44%) Rh-negative. This Rh-negative frequency is notably lower than any recorded previously. Table IX shows that the sexes in each group and the Rh-negatives in both sexes are almost equally distributed. In contrast, the Rh-negative frequencies within each group

Table VIII. Cold-Warm Hemagglutinins in Pregnancy

Tested With	Diluted In	Temperature and Time Incubated	Diluted 1:						1:1 Control Sera		
			1	2	4	8	16	32	64	Rh Pos.	Rh-Hr Neg.
O,M,CcDEe Cells in Albumin	Albumin	37°C for 30 min.	4	4	4	2	1	-	-	4	-
		25°C for 60 min.	4	4	4	3	1	-	-	4	-
		4°C for 5 min.	4	4	4	4	1	/	-	4	-
		56°C for 5 min.	-	-	-	-	-	-	-	4	-
		37°C for 10 min.	4	4	3	1	-	-	-	4	-
O,N,ccdde Cells in Albumin	Albumin	37°C for 30 min.	4	4	4	4	2	1	-	-	-
		25°C for 60 min.	4	4	4	4	3	1	-	-	-
		4°C for 5 min.	4	4	4	4	3	1	/	-	-
		56°C for 5 min.	-	-	-	-	-	-	-	-	-
		37°C for 10 min.	4	4	4	4	1	-	-	-	-
O,M,CcDEe cells in Saline	Saline	As above	All results were neg.						Not Done		
O,N,ccdde cells in Saline	Saline	As above	All results were neg.						Not Done		

very substantially. To determine the statistical significance of these variations, the Chi-square test was applied to the data for groups A and O only, groups B and AB not having sufficient Rh-negatives for such treatment. Analysis reveals that the deviation from the overall expectancy, 0.44%, in Group A is not significant. However, the deviation in group O yields a Chi-square of 6.2 for one degree of freedom and hence a probability of less than 0.02. It therefore seems unlikely that the observed difference is due to chance. Conceivably, a sample containing considerably more Rh-negative individuals might alter present implications.

D^u ANTIBODIES IN PREGNANCY: THEIR ROLE IN IDENTIFYING A RARE D^u GENOTYPE: The anticipated loss of a donor who had routinely provided ccddee (negative control) erythrocytes for Rh studies led to search for a replacement in the same category. Donor S seemed a likely substitute. His red cells did not react with anti C (rh'), anti D (Rh₀) or anti E (rh'') sera but were agglutinated by anti c (hr') and anti e (hr''). He was therefore genotyped cde/cde and designated an rh-negative control. Soon after, it was discovered that he had been genotyped erroneously and that he was not a suitable negative control. This opinion developed from the following observations:

The blood of a woman, gravida 3, was submitted for Rh study. Her first pregnancy had resulted in a hydrocephalic stillbirth and the second in fatal erythroblastosis fetalis. Current red cell analyses classified her as O, ccddee, M, Fy^a-negative, K-negative and her husband as A₁, ccDEe, M, Fy^a-negative, K-negative. Tested with Rh-positive cells, her serum revealed an albumin agglutinin titer of 1:128 and no saline antibodies. However, instead of a negative reaction with erythrocytes from S, unexpected agglutination was observed. This unusual finding provoked further inquiries into the nature of the negative control cells and the maternal serum. The facts uncovered are presented in Table X.

Serum which readily distinguishes Rh-positive from Rh-negative individuals always contains potent anti D (Rh₀) agglutinins. At times it also includes antibodies specific for D^u, a variant of the D antigen. Characteristically, the D^u antibody results from incompatible transfusions, is comparatively weak and, when present, invariably coexists with anti D. Further, anti D^u manifests its specific activity almost exclusively through mediation of the indirect Coombs test. For the present study an anti D serum known to be supplemented by anti D^u was expressly employed.

Table IX. Rh-Negative Distribution in Japanese by Sex and Blood Group

Total Persons Studied	4,541	
Rh-negative		20 (0.44)*
Male	3,075	
Rh-negative		14 (0.46)
Female	1,466	
Rh-negative		6 (0.41)
Group O	1,501	
Rh-negative		13 (0.87)
Male	998 (32.5)**	
Rh-negative		9 (0.90)
Female	503 (34.3)***	
Rh-negative		4 (0.80)
Group A	1,659	
Rh-negative		6 (0.36)
Male	1,145 (37.2)	
Rh-negative		4 (0.35)
Female	514 (35.1)	
Rh-negative		2 (0.39)
Group B	979	
Rh-negative		1 (0.10)
Male	663 (21.6)	
Rh-negative		1 (0.15)
Female	316 (21.6)	
Rh-negative		0 (0.00)
Group AB	402	
Rh-negative		0 (0.00)
Male	269 (8.7)	
Rh-negative		0 (0.00)
Female	133 (9.0)	
Rh-negative		0 (0.00)

* Figures in parentheses indicate percents.

** Percent of 3,075 males; calculated similarly for groups A, B and AB.

*** Percent of 1,466 females; calculated similarly for groups A, B and AB.

Table X shows that the patient's serum exhibited greater anti D^u activity than the known anti D serum. In the absence of a history of transfusions, this observation suggests transplacental isoimmunization of the mother to a D^u factor in the fetus. However, proof of this hypothesis must await delivery of the infant and analysis of its erythrocyte antigens.

Although the presence of D^u in the husband's cells, shown to be ccDEe, may be predicated on the reactions observed and on consequent genetic implications, direct demonstration of this factor by laboratory methods has not been successful. Apparently the D-D^u complex in his cells prevented distinguishing Anti D^u from anti D activity of the maternal serum. Absorption procedures were applied to effect separation but failed.

The table contributes additional evidence of note. S, heretofore considered rh-negative, is patently not in that classification. He possesses the D^u intermediate as a separate entity and not in combination with C (rh') or with D (Rh₀) and E (rh'') as is postulated for the husband. Such occurrence of D^u by itself is rare and, according to the British investigators, appears in but one of 6,666 persons.

Table X. Antibody Detections Using Erythrocytes: A. In Albumin;
B. Treated with Papain; C. Prepared for In-
direct Coombs Test

<u>Erythrocytes</u>	<u>Patient's</u>	<u>Serum</u> <u>Known Anti D*</u>	<u>Normal</u>
A. In albumin:			
Known: O,CcDe,M	4/	4/	Neg.
Known: O,ccddee,N	Neg.	Neg.	Neg.
S : O,ccddee,M	1/**	Neg.-Trace**	Neg.
B. Papain-treated			
Known: O,CcDEe,M	4/	4/	Neg.
Known: O,ccddee,N	Neg.	Neg.	Neg.
S : O,ccddee,M	1/	1/	Neg.
C. For Coombs Test***			
Known: O,CcDEe,M	4/	4/	Neg.
Known: O,ccddee,N	Neg.	Neg.	Neg.
S : O,ccddee,M	3/	1/	Neg.

* Also contained anti D^u.

** Appeared only after incubation at 37°C for 30 min.

*** Most effective for demonstrating D^u antigen.

FAR EAST MEDICAL RESEARCH UNIT

INTRODUCTION: It is equally true of military and civilian medicine that systematic and planned research must be continuously and vigorously pursued if progress is to be maintained. Many problems of military medicine are rarely encountered in civilian medicine. It is therefore essential to conduct medical research during wars, and since the military patient can seldom be moved to a well-equipped, fixed, research institution, it becomes necessary to move the research personnel and equipment to the patient.

The biggest medical problem of war is the treatment of trauma and its complications. To meet this problem, a better understanding of the physiological changes produced by trauma is essential. Infections, hemorrhage, vascular repair, renal insufficiency, blood volume changes, plasma expanders, analgesic and anaesthetic agents and effective transportation of the acutely injured present challenging problems.

The Far East Medical Research Unit was organized on 10 March 1952, by Special Order Number 27, Headquarters, Japan Logistical Command. Its basic mission is "To conduct, coordinate, supervise, and direct such medical research within the Far East Command as is directed or approved by appropriate authority. To support, assist, and advise those special teams entering the Far East Command for the investigation of special medical problems beyond the capacity of units and personnel available in the Command".

Those persons permanently assigned to the Far East Medical Research Unit are so closely integrated with the 406th Medical General Laboratory in the conduct of research that it is not practical to separate the projects of the two organizations. The work of most of the permanent staff of the Far East Medical Research Unit have therefore been included in the appropriate sections of the report for the 406th Medical General Laboratory.

Several semi-permanent research teams have operated on a temporary duty basis with the Far East Medical Research Unit. These teams have worked primarily in Korea, with the support of the various departments of the 406th Medical General Laboratory.

Each individual or small group of persons within a research team has set out to investigate a specific facet of a medical problem. Most of these persons arrived with a previously prepared protocol and departed the Far East Command when this protocol or a modification of it was completed. From time to time, recognized civilian consultants visited and assisted the teams in the field, contributing greatly to the success of the research program.

A review of the projects conducted by the Far East Medical Research Unit demonstrates the practicability of field research. This type of research goes hand-in-hand with the best type of therapy and has as its sole purpose the provision of better medical support to the soldier. All studies were designed to aid in treatment and were carefully planned and executed to assure that no interference or delay in the normal care and evacuation of patients occurred. The results indicate that field research was a valuable adjunct and that such research should continue at each level of the evacuation chain.

This section of the annual report briefly summarizes the preliminary observations, theories, and recommendations of the teams and of the visiting advisors. While a few of these reports have since been prepared for publication, most are of a preliminary and continuing nature and must not be interpreted otherwise. The following teams have made memorable contributions to field research and have been supported by the Far East Medical Research Unit:

Epidemic Hemorrhagic Fever Research Team
Surgical Research Team
Wound Ballistics Research Team
Body Armor Research Team
Psychiatric Research Team

EPIDEMIC HEMORRHAGIC FEVER: The studies of Epidemic Hemorrhagic Fever have been primarily limited to investigations into the epidemiology, etiology, clinical management, physiology, and pathology.

Epidemiology - Hemorrhagic Fever is characteristically rural rather than urban and cases are for the most part sporadic and not closely associated in time or space with other cases. Clusters of cases have occurred, usually limited to part of a unit, such as a platoon, a single tent, or the crew of a single heavy weapon, without affecting other members of the parent organization. In such clusters, the disease seems to have been contracted at about the same time by many patients, and other cases did not follow. The patchy, sporadic incidence finds many analogies in the epidemiology of scrub typhus. A prominent feature of the epidemiology is the occurrence of two distinct seasonal peaks, in May-June and October-November.

Doctor Ross L. Gauld, Epidemiologist to the Hemorrhagic Fever Research Team, conducted several surveys following outbreaks of hemorrhagic fever involving men working or living closely together (1,2,3). From these investigations, it was concluded that the incubation period was usually from 10 to 16 days, with considerable variation on each side; that the spotty distribution was consistent with an arthropod vector which did not normally travel over great distances. He therefore indorsed the use of insect repellent and the continuation of entomological investigations to characterize the arthropod vector.

Etiological Agent - Interest in the etiology of hemorrhagic fever continued during 1952 (4). Studies concerned with the recovery of a specific agent were directed primarily by members of the Armed Forces Epidemiological Board, with the aid of personnel and equipment from the Department of Virus and Rickettsial Diseases, 406th Medical General Laboratory. Doctors J. E. Smadel, K. C. Smithburn, and Q. M. Geiman were responsible for studies made during the spring and summer of 1952 (5). Dr. J. Warren and Miss E. Jackson represented the Commission on Hemorrhagic Fever, Armed Forces Epidemiological Board, during the fall. The following report is based on observations made by these consultants, and the staff of the 406th Medical General Laboratory. More detailed information is to be found in the official reports of the Commission on Hemorrhagic Fever to the Armed Forces Epidemiological Board.

Japanese and Russian investigators have had reasonably broad experience with this disease. Limited experiments by Smorodintsev and co-workers (6) showed that ultrafiltrates of blood and urine obtained before the 5th day of disease were infectious for humans; both Japanese and Russian workers failed either to establish obvious disease in laboratory animals, or to maintain it by serial passage, although both groups thought that wild rodents (Apodemus agrarius and Microtus michnoi) could be infected by the inoculation of blood or urine obtained from acutely ill patients.

While attempts at isolation of an agent in a wide variety of experimental animals had been made in this laboratory in 1951, blood passage had been made in only a limited number of cases. The initial approach in 1952 was based upon the hypothesis that an infectious agent might be so adapted by serial passage in some laboratory animal that it would produce obvious disease. This was developed along the following lines: Blood (and in some instances bone marrow) was obtained from suspected patients within 48 hours of the onset of fever, and was immediately inoculated intracerebrally and intraperitoneally into hamsters, Microtus pennsylvanicus, and 2 strains of albino mice. Cynomolgous monkeys were inoculated subcutaneously, intravenously, and intraperitoneally. Generally, 4 passages were made at 4-6 day intervals in ordinary white mice (406 strain) and in primates, and a variable

number of hamsters and *Microtus*. Inoculated cynomolgi were bled on the 5th, 10th, and 15th day postinjection, and each blood inoculated into the same passage monkey, and into different sets of albino mice. As indicated by illness, passages were made from the originally inoculated animals of the special mouse stock (BS-VS). Additional inoculations were made into suckling mice, young guinea pigs, and embryonated eggs. Inoculated animals over and above the capacity of the 406th Medical General Laboratory were shipped to laboratories in the United States for further follow-up and passage. Rodents received by the Army Medical Service Graduate School in Washington were passaged at 12-day intervals four times. Material from this last passage was inoculated into embryonated eggs. Injected cynomolgi were sent to Dr. John Paul at Yale University and passages were made into rhesus monkeys and chimpanzees.

The inoculation of material from over 40 patients subsequently confirmed to have hemorrhagic fever has failed to establish a transmissible disease in laboratory animals which did not later prove to be due to an infectious agent naturally occurring in laboratory animals. Tyzzer's Disease (7) was encountered on several occasions in hamsters and in mice, and an unidentified transmissible infection involving the liver was observed at least twice.

Certain clinical features of hemorrhagic fever resemble those of rickettsial infections in man, and in cattle. Stained impression smears of tissue obtained at autopsy were searched for rickettsia-like organisms. Six series of stained films were adequate for study. In 3 of these, intracellular rickettsia-like bodies were found in endothelial cells from the pituitary gland, inferior vena cava, adrenal glands, and splenic capsule. The cytoplasmic distribution of these bodies was studied by Dr. J. Warren, who suggested them to be distinct from normal or abnormal granules of polymorphonuclear leukocytes, mast cells or other granulocytes. These bodies were not difficult to find in smears from patients dying before the 5th day of disease, but were scarce or absent in patients dying 8 days or more after onset. The observed granules were more apparent in Giemsa stained preparations than in films stained by the Machiavello technique.

The finding of these inclusion bodies was the basic reason for using embryonated eggs more extensively in continuing attempts to isolate the etiological agent of epidemic hemorrhagic fever. Fresh blood or autopsy material from 18 confirmed cases of the spring epidemic were inoculated into fertile hen's eggs. Approximately 10 blind serial passages were made in the same host. Stained smears of embryonic tissues were made at harvest without consistently demonstrating rickettsia-like bodies. No identifiable infectious agent was recovered.

Work during the fall outbreak was concerned primarily with intensifying the study of the rickettsia-like inclusions seen in a few cases dying late in the spring epidemic. The spectrum of laboratory animals inoculated was extended to include pigeons, albino rats, dogs, and cats, but major emphasis was placed upon the inoculation of embryonated eggs by different routes with blood obtained within the first 48 hours of disease, and with autopsy tissue. Stained impression smears obtained at autopsy were studied for intracytoplasmic inclusions.

Passages were not usually made unless signs of illness were apparent. A few rat and hamster lines were blind passaged at 21-25 day intervals to provide living potentially infected animals for transportation to the United States. All animals which were sacrificed for any reason were autopsied, and impression smears of tissues were stained. To date no specific disease has been reproduced in laboratory hosts, nor has it been possible to consistently demonstrate in any species intracytoplasmic inclusions resembling those seen in fatal human cases.

Serial passages from 8 blood specimens taken early in the disease, and from 10 fatal cases have been maintained in eggs. Routes of inoculation included yolk, amniotic and allantoic sacs. Stained smears of passage materials from these egg lines

were observed at time of passage without the conclusive demonstration of rickettsia-like bodies. No demonstrable new organism has been observed in these egg lines to date.

The intracytoplasmic endothelial inclusion bodies were again seen in tissues obtained from fatal cases of epidemic hemorrhagic fever in the fall. It is currently the impression that such granules are most intimately associated with endothelial cells of the large vessels; more critical observation is required to confirm this. Whether or not these inclusions represent an "elementary body" form of the infectious agent itself, or some product of altered cellular metabolism, is unknown; definition of this point will depend upon the successful reproduction of these bodies by some cultural technique.

Isolation studies are continuing in Korea, Tokyo, and Washington. Various insects are used as primary experimental animals in the attempt to isolate the agent. This approach is based on the successful cultivation of various micro-organisms, and notably the pathogenic rickettsiae, when inoculated into the body cavity of insects. This work has just begun.

Entomologic Investigations - Entomological investigations of hemorrhagic fever have produced information pointing rather clearly toward chiggers (the parasitic larval stage of mites) as the probable vector of this disease. Although confirmation of this presumption must await successful isolation and manipulation of the etiologic agent, the accumulated evidence may be summarized as follows (8,9,10):

In scrub typhus, a chigger-borne disease, there occurs a characteristic "spotty" distribution within broad areas of infection, due to the fact that chiggers normally occur only in the immediate vicinity of the site of oviposition of the adult female. Further, there is a close correlation between the seasonal peaks of scrub typhus and the seasonal incidence of vectors. In Korea the seasonal abundance of chiggers corresponds well with the spring and fall peaks of hemorrhagic fever. Chiggers are common in the spring, relatively uncommon during the summer months, become very abundant in the fall from September through November, and decline in December. The most abundant chigger species in Korea are related to the known vectors of scrub typhus, a group that ordinarily does not produce itching when infecting man. It is therefore considered probable that these minute forms could attack man and consistently escape detection. Chiggers greatly outnumber other ectoparasites such as laelaptid mites in the known endemic areas throughout most of the year, and are the outstandingly dominant group during periods of presumed hemorrhagic fever infection. Furthermore, they are very hardy, capable of survival under adverse conditions, and of activity at temperatures much lower than most other parasitic arthropods. The life cycle of trombiculid mites is intimately associated with soil or soil surface litter, and the length of time that elapses following oviposition, before the appearance of the parasitic larval stage, is sufficiently great to permit marked changes of the environment from that required by the adult mite. The larval stage may remain dormant for some time in the locale in which it hatched, awaiting the appearance of a suitable host. For these reasons, persons who are brought into intimate contact with the soil during periods of high chigger abundance would be particularly exposed to contracting chigger-borne diseases. The epidemiologic evidence points toward this sort of transmission.

The investigational approach to the entomology of hemorrhagic fever during 1952 was largely through trapping of rodents and other small animals in areas of known endemicity, and analyzing their parasitic fauna with respect to numbers and kinds of parasites present. The four rodents receiving particular attention in this program were Apodemus agrarius, Microtus fortis pelliceus, Clethrionomys rufocanus regulus and Cricetulus triton nestor.

The chiggers on Apodemus agrarius will serve to illustrate the seasonal fluctuations encountered in 1952. The average number of chiggers per mouse (chigger index) in early May was 14. By the end of June the index had dropped to one, and

during July and August it was less than one. However, by the end of September the index was 30 and by mid-October had reached the maximum - an average of 80 chiggers per Apodemus. The index remained high in November and started to fall rapidly in December so that by the end of that month the average number of chiggers per Apodemus was 13.5. The same general seasonal trend is shown by the chiggers of the reed vole, Microtus fortis, the red-backed vole, Clethrionomys rufocanus and by the hamster, Cricetulus triton, although these animals consistently carried more chiggers than did Apodemus during the spring, summer, and fall.

The commonest species of chigger encountered in Korea during the period May-December 1952 was Trombicula (Leptotrombidium) pallida. T. (L.) orientalis was second most abundant, with T. (L.) palpalis also well represented. T. pallida exhibited an interesting seasonal variation in abundance of the larval form, it being the most prevalent chigger in May and again in September and October. Only a small percentage of the total chiggers collected in November were pallida, and the species was not found in December. The maximum peaks of abundance of pallida larvae preceded by two to four weeks the normally expected maxima of hemorrhagic fever cases, and the decline of cases follows the decline of abundance of the species by a similar period. The four species of wild Korean rodents share the trombiculid mite fauna, although some host preference is indicated. The chigger found most commonly on Apodemus and on Microtus was Trombicula (Leptotrombidium) pallida - 40 per cent of all specimens examined from Apodemus and 61 per cent of all the chiggers taken on Microtus were this species.

Laelaptid mites are parasitic in all their active stages of development, but none of the Korean species encountered exhibited seasonal peaks of abundance that could be correlated with the incidence of hemorrhagic fever. They were far less abundant than trombiculid mites. Fleas and ticks, the other ectoparasites of rodents likely to be involved in disease transmission, were relatively scarce.

Proof regarding the definitive role of any of the potential arthropod vectors of epidemic hemorrhagic fever must await the discovery of a susceptible laboratory host for the agent and its use in the usual isolation and transmission studies. Entomological and epidemiological observations provide information which indicate a group of arthropods as vectors of this disease; these are the ground-inhabiting arthropods of limited mobility, such as chiggers, laelaptid mites, fleas, and ticks.

Clinical Aspects - Detailed description of the symptomatology of hemorrhagic fever in its various phases can be found in individual reports of consultants and in the reports of the Commission on Hemorrhagic Fever, Armed Forces Epidemiologic Board. Only those brief descriptions necessary for continuity are contained in this report.

Febrile Phase: An irregular high fever is characteristic of the first four days. The usual early symptoms are indistinguishable from many infectious diseases beginning with headache and general malaise. Malaise is prominent and is associated with restlessness, myalgia, and lumbar backache. Temperature rise is rapid, and reaches 102°F or more, on the first day, in many cases. Irregular high fever (101°F to 106°F) may persist until the onset of hypotension. Anorexia occurs early and may progress to nausea and vomiting on the third or fourth day of illness, particularly in over-dehydrated patients. Thirst is prominent and may lead to excessive fluid intake.

The two most typical physical findings are facial flush and injection of the conjunctivae and palate. The flush is a diffuse erythema involving the face, neck, and upper anterior chest and resembles a first degree sunburn. The conjunctival and palatal injection is fine, diffuse, reticulated, and not associated with a purulent exudate.

Hypotensive Phase - Significant clinical shock appears in approximately 25 per cent of hemorrhagic fever patients. It is commonest on the fifth or sixth day but may appear as early as the third. It lasts from a few hours to several days. Significant hypotension is absent in the remaining 75 per cent of the cases and these patients pass directly into the oliguric phase.

Physical findings in the shock stage suggest widespread capillary damage. The diastolic pressure is maintained initially, thus readings such as 100/80, 90/80, and 86/82 are typical. A thready, weak pulse usually persists, but in severe cases blood pressure and peripheral pulse are unobtainable, the neck veins collapse, heart sounds are weak and rapid, with an apical rate of 150/minute or more. Conjunctival edema increases and petechiae appear in increasing numbers.

Oliguric Phase - Urinary output decreases sharply at the end of the febrile phase and oliguria or occasionally anuria persists for two to six days. The severity of the renal phase is independent of the hypotensive period and severe azotemia may be seen in patients who never show hypotension. The symptoms of this phase are those of acute azotemia. Often the patients become irritable, restless, and sometimes combative. The blood pressure rises during oliguria, usually to mild hypertensive levels but it may reach 200/120 and be associated with encephalopathy and convulsions. Bradycardia is common. Uremic frost, pericarditis, diarrhea, and the hyporeflexia and weakness of hyperkalemia may occur in more severe cases.

Convalescent Phase - The oliguric phase terminates in a profuse diuresis, usually about the tenth day of illness. Because of impaired renal concentrating ability, polyuria persists. The concentrating ability of the kidneys gradually improves, returning to normal in five to six weeks.

Disturbed Physiology in Hemorrhagic Fever - Doctor David P. Earle, Associate Professor of Medicine, New York University College of Medicine, New York, consultant to the Surgeon General, visited the Hemorrhagic Fever Center in a consultant's and advisor's capacity (11). He believes that many of the phenomena can be interpreted on the basis of accepted physiologic concepts which will offer a rational basis for the management of the patient. There are a variety of nervous, humeral, and metabolic abnormalities which undoubtedly contribute to, or play an important role in, the pathogenesis of the clinical manifestations of hemorrhagic fever. Where these are more clearly understood management of the disease may be greatly simplified. Some of the most prominent are:

- (1) Arteriolar Dilatation in Hypotensive Phase.
- (2) Loss of Plasma in Hypotensive Phase.
- (3) Return of Plasma to Vascular System.
- (4) Trapping of Erythrocytes in Hypotensive Phase and into Diuretic Phase.
- (5) Reduction of Available Vascular Space in Hypotensive, Oliguric, and Early Diuretic Phases.
- (6) Relative Hypervolemia in Oliguric and Early Diuretic Phases.
- (7) Renal Failure in the Latter Phases of the Disease.

A wide variety of therapeutic agents and methods have been tried in the management of these patients. In general, it has been determined that relative dehydration with careful attention to blood volume, electrolyte balance and the maintenance of blood pressure are the most useful therapeutic methods. During the spring epidemic, Trendelenberg's position and pressure bandages on extremities became standard treatment during shock.

In the early fall, Doctor W. Barry Wood, Washington University Medical School, made a brief but intensive study of the disease (12). Since blood vessels were dilated and engorged with little or no extravasation of red cells into surrounding tissue, and since frequent early symptoms include dizziness or syncope, and a flushed warm skin, he postulated that the vasoconstrictor nor-adrenalin might be valuable in this disease.

While subsequent experience has indicated that some patients become refractory even to huge doses of this agent, and that it is apparently ineffectual in preventing the development of shock when given to patients early in the disease, it is generally conceded to be an additional and powerful tool in maintaining blood pressure during the shock phase (13).

Body Water Compartments - In an effort to assess the shifts of fluid between the vascular compartment, interstitial compartment, and intracellular compartment, simultaneous measurements of the volumes of distribution of Evans' blue dye, sodium thio-sulfate, and deuterium oxide were done in various phases of hemorrhagic fever (14). The Evans' blue dye studies were done by 1st Lt. Robert Giles of the 8228th Mobile Army Surgical Hospital. The deuterium oxide and sodium thiosulphate studies were undertaken by Major Marion E. McDowell and Lt. Frank Furth, both of the Army Medical Service Graduate School. Much further work is required before any interpretations of these investigations can be made.

Renal Pathophysiology - Major McDowell, along with Captain Herman Froeb of the 8228th Mobile Army Surgical Hospital undertook an investigation of the renal pathophysiology by measurement of the glomerular filtration rates and minimum effective renal plasma flow. Their studies revealed normal or high renal plasma flows and normal or high glomerular filtration rates during the early febrile phase in the six patients they were able to follow carefully. This has not disclosed the fundamental cause of the renal lesion in hemorrhagic fever, but compensatory ischemia does not appear to play an important role.

Hematologic Observations - Lt. Frank Furth of the Army Medical Service Graduate School (16) carefully observed hematologic changes during the course of disease in 29 patients with hemorrhagic fever. He noted a rather severe leucocytosis with a marked shift to the left in the early stages, with the appearance of atypical lymphocytes on the 4th or 5th day. The appearance of these cells was concomitant with an increasing lymphocyte count. Failure of the atypical lymphocytes to appear indicates a grave prognosis.

The hematocrit was closely followed in these cases and appears to be correlated with the clinical course. Slowly increasing levels are seen during the febrile stage with a sudden rise at the beginning of the hypotensive phase. Hematocrit levels of 60 and 65 are common. As blood pressure rises the hematocrit falls. This continues during oliguria and normal levels are reached in the diuretic phase.

Coagulation time and prothrombin levels were usually normal though clot retraction may be poor. Prolonged bleeding time depressed platelet count, and increased capillary fragility are characteristic.

P³² Blood Volumes - Twenty patients were studied by Major Edward Langdon using a modification of the Reeve-Veall radioactive phosphorous (P³²) red blood cell tag technique for the determination of blood volumes in the various phases of hemorrhagic fever (17).

Although the number of patients in each phase is considered too small to be conclusive - and although an accurate picture of blood volume changes requires a technique which allows one to follow an individual patient through all phases of his illness, trends can be established from this series.

The plasma volume and thus total blood volume, fall in the febrile stage, while the red blood cell volume remains within normal limits. In the shock phase, the red blood cell volume is decreased. In the oliguric phase, the red blood cell remains low, while the plasma volume is low to normal - with the total blood volume remaining low to low normal. In the diuretic phase, the red cell volume remains low - whereas the plasma volume is normal to high and the total volume is normal to high.

Comparative Pathology - 1951 and 1952 - A comparative study of the 1951 and 1952 autopsy cases was undertaken by Lt. Colonel Arthur Steer to determine whether any consistent and recognizable change in pathology had occurred between the two years (18). For this purpose the cases were arranged by year according to the stage of disease in which death occurred, i.e., shock, oliguric or diuretic. The clinical history, gross and microscopic pathology were then reviewed carefully to demonstrate similarities or differences. In general, microscopic changes were graded in severity from one to four with one representing the minimal changes observed and four representing maximal.

There was a slight overall decrease in mortality in 1952, as is evidenced by a death rate of 6.4% in the fall outbreak of 1951, compared to 4.9% during the entire year of 1952. This slight overall decrease represents a rather sharp decline in the expected mortality rate during the oliguric phase. This is shown in Table I and probably represents the effect of fluid restriction in the therapeutic regimen employed in 1952.

Table I. Number of Deaths Expected in 1952 Based on 1951 Rates

<u>Stage of Disease At Time of Death</u>	<u>Number Expected</u>	<u>Number Which Occurred</u>	<u>Variation</u>
Shock	25	23	- 2
Oliguria	33	14	- 19
Diuresis	10	13	+ 3
	—	—	
Total	68	50	

Comparative weights of organs from patients dying in the various stages of hemorrhagic fever are shown for the two years in Table II. There is a consistent difference in gross weight of the organs during the two years, except for the liver.

Table II. Average Weights of Organs by Stage of Disease, 1951-1952

<u>Organ</u>	<u>Year</u>	<u>SHOCK Av. Wt.</u>	<u>No. Pts.</u>	<u>OLIGURIA Av. Wt.</u>	<u>No. Pts.</u>	<u>DIURESIS Av. Wt.</u>	<u>No. Pts.</u>
Lungs	1951	1059	16	1598	25	1349	9
	1952	778	16	1273	9	1222	10
Heart	1951	373	17	354	28	333	9
	1952	317	16	316	8	317	10
Spleen	1951	267	17	244	28	199	9
	1952	230	16	154	8	123	11
Kidneys	1951	511	17	537	28	506	9
	1952	431	16	466	9	407	11
Liver	1951	1761	17	1925	28	1869	8
	1952	1694	16	1933	9	1922	10

While there was a decreased incidence of pulmonary edema in 1952, especially in those dying during the diuretic phase, there was a counterbalancing increase in pneumonia as revealed by histological examination. The degree of pneumonia was graded one to four and is shown in Table III. Grade one represented small foci of broncho-pneumonia, three and four being diffuse consolidation involving an entire histologic section. Four differed from three only in that suppuration was present.

The average weight of the heart in 1952 was normal in all stages of disease (Table II). This was in rather sharp contrast to the findings in 1951, when the heart weight was increased in the shock phase, progressively decreasing in the later stages.

A significant observation when the cardiac sections were reviewed was that a very mild but almost constant change could be observed which was diagnostic. This change was described in 1951, but no particular significance was attributed to it

Table III. Average Severity Of Pneumonia As Seen Histologically By Year
And Stage Of Disease

<u>Year</u>	<u>Shock</u>	<u>No. Cases</u>	<u>Oliguria</u>	<u>No. Cases</u>	<u>Diuresis</u>	<u>No. Cases</u>
1951	0.33	16	0.95	25	1.89	9
1952	0.17	16	1.82	9	2.27	10

because of its minimal nature. This change consists of a mild infiltration of cells into an edematous layer immediately beneath the intact and unaltered endothelium of the endocardium. The infiltrate consists principally of various mononuclear cells with only a rare polymorphonuclear leukocyte. It is only a few cells thick and was found in all chambers and even occasionally on the valves. These infiltrates could be traced between muscle bundles into the interstitial tissue. Similar infiltrates were frequently seen beneath the endothelium of the aorta and occasionally of the coronary arteries.

There was little variation in the incidence of these observations between 1951 and 1952, but there was a decreasing prominence from the shock phase thru the diuretic phase. This is shown in Table IV.

Table IV. Cardiac Changes By Stage Of Disease - 1951 and 1952 Combined

<u>Histologic Finding</u>	<u>Shock</u> <u>(39 Cases)</u>	<u>Oliguria</u> <u>(32 Cases)</u>	<u>Diuresis</u> <u>(17 Cases)</u>
Subendocardial Edema	100%	82%	53%
Subendocardial Infiltrate	97%	78%	65%
Myocardial Infiltrate	64%	63%	41%

Some "hemorrhage" was seen in the right auricle in all cases. It tended to occupy the edematous endothelial layer and frequently was massed so that cell outlines could not be distinguished. No cellular reaction was found to this extravasated blood, even in the cases of longest duration. Considerable variation in the size of erythrocytes was observed, extremely small erythrocytes being frequently found. These small erythrocytes were observed in other areas of "hemorrhage" as well.

The renal lesions seen in 1952 were much less severe than those of 1951 but the fundamental changes described in 1951 by Steer and Hüllinghorst were present (19, 20). Reexamination of the cortico medullary "hemorrhage" revealed that engorged, dilated vessels made up the "hemorrhage" in cases of 5 or less days duration. In cases of longer duration distinct vascular channels were not found but special stains revealed delicate fibers suggesting an extremely dilated sinusoidal pattern. True hemorrhage seldom existed and it is now believed the appearance of hemorrhage is due to vascular dilatation and diapedesis of red cells.

The pathogenesis of the medullary necrosis is still obscure, though there is some evidence that it may be secondary to anoxia. Medullary necrosis was somewhat less severe and less frequent during 1952. This is indicated in Table V which compares both incidence and severity for the two years. Severity has been graded one to four as in other tables.

Changes in the anterior pituitary assume a somewhat altered relation when considered according to stage of disease. Necrosis was present in all cases dying in oliguria and in 17 of 18 dying in the diuretic stage. It was less frequent in the deaths occurring in the shock stage. There was little difference in severity of the necrosis between the two years (Table VI).

Table V. Incidence And Severity Of Medullary Necrosis By Year And Stage Of Disease

	SHOCK		OLIGURIA		DIURESIS	
	1951	1952	1951	1952	1951	1952
Number of Cases	20	23	27	14	9	13
Necrosis Present	25%	4.3%	51.9%	35.7%	66.7%	21.5%
Degree of Involvement	0.45	0.04	1.48	0.71	2.11	0.62

Table VI. Necrosis, Anterior Lobe Of Pituitary By Year And Stage Of Disease

	SHOCK		OLIGURIA		DIURESIS	
	1951	1952	1951	1952	1951	1952
Number of Cases	17	19	21	9	6	12
Necrosis Present	53%	63%	100%	100%	100%	92%
Degree of Involvement	1.2	1.0	3.1	2.9	2.5	1.9

There was a striking difference in adrenal necrosis between the two years. This was especially true in the oliguric and diuretic stages (Table VII).

Table VII. Necrosis Of Adrenal By Year And Stage Of Disease

	SHOCK		OLIGURIA		DIURESIS	
	1951	1952	1951	1952	1951	1952
Number of Cases	20	22	29	13	8	13
Necrosis Present	15%	23%	35%	0%	0%	15%
Degree of Involvement	0.5	0.6	1.1	0	0	0.4

It is difficult to evaluate the observed differences in histological changes during the two years experience. It is believed however that most of these differences probably reflect the difference in therapy between 1951 and 1952, rather than an actual change in the character of the disease. Considerably more study of the histological variations is planned.

SURGICAL RESEARCH TEAM: The Surgical Research Team was organized at the Army Medical Service Graduate School and sent to the Far East in December, 1951. The broad purpose of the team was the study of the combat casualty to seek means for further reduction in both mortality and permanent incapacity.

Upon arrival in Japan the team was assigned to the Far East Medical Research Unit for administrative and logistic support. It was then placed on indefinite temporary duty with Headquarters, Eighth Army. In Korea the team was further divided between several hospitals for messing, housing, and clinical support. The support received from the Eighth Army Surgeon and his staff, from the administrative and professional staff of the 8209th Mobile Army Surgical Hospital, the 11th Evacuation Hospital, and 8055th Mobile Army Surgical Hospital was at all times of the highest quality.

Resuscitation Of The Severely Wounded - One of the greatest problems in the treatment of the severely wounded is resuscitation. Therefore, this was the first problem undertaken and has required the major portion of the time and personnel during 1952. Many of the projects under this heading are still in progress and only a summarization of results is possible at this time. (21)

With injury, six things happen to a man:

- (1) Mechanical defects may occur which threaten life or function.
- (2) Tissue is destroyed.
- (3) The defense against bacteria is broken.
- (4) Blood is lost.
- (5) Pain and emotional stimulation occur.
- (6) A series of metabolic-endocrine changes are set in motion.

The evaluation and correlation of any of these defects constitute the problems of resuscitation and are discussed below.

Mechanical Defects May Occur Which Threaten Life Or Function - If these defects affect a vital function, as for example, an open wound of the chest, cardiac tamponade, or respiratory obstruction, emergency treatment may well determine the immediate fate of the patient.

Tissue Is Destroyed - It is well known that a high velocity missile produces tissue destruction far beyond its immediate pathway. Cavitation occurs over a wide area and consists of a series of cavities, each bounded by a fascial plane, rather than a single cavity. As bullets, metal fragments, or glass strike the tissue, each cell becomes a secondary missile, traveling at right angles to the bullet. Bone fragments may tear a wound of exit the size of one's fist although the primary missile was quite small. Wounds of entrance and exit do not indicate the extent of damage in the intermediate tissues. Damage extends laterally beyond the borders of the immediate perforation. The responsibility of the surgeon is to explore each fascial plane, to remove the dirt, cloth, metal and tissue fragments and then to excise the devitalized tissue along the lateral margins. The surgeon accomplishes this in a few minutes and we call the process debridement. Nature requires several weeks and we call the process sloughing.

The Defense Against Bacteria Is Broken - Immediately after injury, the battle casualty receives 300,000 units of penicillin and a booster dose of toxoid. These are given at the Battalion Aid Station. A series of 100 patients was studied upon arrival at the forward hospital approximately three and one-half hours after wounding (22). Contamination of the wound was universal. Clostridia were present in approximately two-thirds of the wounds. Clostridia perfringens was present in approximately one-third. In spite of this contamination, life endangering infections, except in those patients with renal insufficiency, are almost never seen in the battle casualty under present conditions in Korea. This is probably due to the relatively light flow of casualties permitting early and adequate debridement and to the early and consistent use of antibiotics. Gas gangrene has been seen in only about one in a thousand casualties, has rarely proved fatal, and has occurred in ischemic, non-viable extremities in which adequate debridement had not been achieved. Tetanus has not been seen. Peritonitis as a cause of death has been infrequent. Although the defense against bacteria has been broken, early surgical therapy and antibiotics have proved quite efficient. Never before have battle casualties had so little to fear from infection. The short time lag of three and one-half hours between wounding and admission is stressed.

The last three factors, blood loss, pain, and metabolic-endocrine response are so interwoven as to be inseparable in their consideration. Severe pain is unusual in the battle casualty. It occurs, but is infrequent. The need to administer drugs for the relief of pain is therefore infrequent.

A Series Of Metabolic-Endocrine Changes Are Set In Motion - These changes are a response of the autonomic nervous system, of the adrenal gland, of the wound, and of other mechanisms. Defects in the adrenal cortical responses have not been discovered.

Blood Is Lost - The loss of blood and the responses of the autonomic nervous system are the two most important factors in determining the immediate fate of most patients. Our work has confirmed the results of others that a man can lose approximately 25% (about 1250 cc) of his blood volume before his blood pressure falls. A loss of 40% (about 2000 cc) is associated with a marked decrease in blood pressure.

Out of the interplay between blood pressure and the autonomic response emerge three syndromes. Using primarily the blood pressure as the baseline, there are the syndromes of compensation, over-compensation, and decompensation.

Compensation: With a blood loss of 10 to 20%, the autonomic nervous system can produce a vasoconstriction and an increased cardiac rate so that a normal blood pressure will be maintained. The casualty is pale, dry, and usually has a rapid pulse of decreased volume. It is of the utmost importance for the surgeon to recognize this state and to replace the blood volume deficit before any other therapy is undertaken. Morphine or Demoral may cause a sharp drop in pressure. Anesthesia will convert this incipient shock into profound shock. Anesthesia, by blocking the compensatory response, changes compensation to decompensation. Passive movement may have a deleterious effect, particularly if it is accompanied by changes in posture of the patient. If the casualty is transferred on a litter from the receiving ward to the x-ray machine twenty feet away, a drop in pressure is a common observation. On two occasions such patients have suddenly died and autopsy has failed to demonstrate any basis for death. The primary responsibility of the forward surgeon must be to stop hemorrhage. His next responsibility is to replace the blood volume deficit. The less the patient is moved before this replacement is accomplished, the better his chance for survival. "Transfuse them where they lie" should be added to the old adage, "Splint them where they lie".

Overcompensation: This is a syndrome characterized primarily by hypertension. It is associated with a blood loss of 10 to 25% and usually results from severe fractures of long bones associated with considerable destruction of muscle tissue. In this syndrome pain is usually severe and may be the etiological factor. Whatever the etiology, the hypertension is maintained through the mechanism of the autonomic nervous system.

The pressure may rise as high as 240 mm systolic but it is characteristically around 150-160 mm mercury. A sharp drop in pressure follows the administration of ganglionic blocking agents. Pentothal, routinely used in Korea, produces a profound drop in pressure. Narcotics, intravenously, may or may not produce a very gradual return to a normal pressure.

Therapy should consist of immobilization, analgesia, and replacement of the blood volume deficit. Anesthesia should await stabilization of the reaction. Stabilization is used instead of subsidence as the hypertension may persist for a long (undetermined) period of time. This syndrome represents one of the very few instances in which morphine has a place in resuscitation, before arrival at the forward hospital.

Decompensation: This term is used as a syndrome of shock in its accepted sense. With a loss of over 25% of blood volume, the body is unable to maintain a normal blood pressure. This does not imply that the autonomic nervous system is not active. It may be quite active as indicated by the fact that following partial replacement of his blood volume deficit, a hypertension may develop.

Anesthesia blocks the responsiveness of the patient and at this critical point is an additional injury of critical importance. As has been pointed out, the autonomic nervous system is active. Since anesthesia inhibits this response, it furthers the injury. Vasoconstrictors play their chief role in resuscitation at this point.

Massive transfusion may be necessary to restore normal blood pressure at this time, but based on observation of 4500 battle casualties whose evacuation time averaged three and a half hours, normal blood pressure can always be restored prior to anesthesia if there is no central nervous system injury or there is no continued loss of blood. During or following anesthesia, this statement becomes invalid.

A review of the experiences with some of the most severely injured patients demonstrates several points of interest (23).

Thirty casualties were studied who, on admission, had an imperceptible blood pressure. Table I demonstrates two observations; one, that the prognosis of the casualty with a severe abdominal wound is more grave than the casualty with a severe extremity wound; and, two, that the amount of blood required for resuscitation is a better indication of prognosis than is the admission blood pressure. Of ten patients with abdominal wounds, four died of continuing hemorrhage and three died in the absence of continuing hemorrhage. Of the 14 patients with extremity wounds, only one died. Thus, the overall mortality of the abdominal wounds (70%) is ten times that of the extremity wounds (7%) in this small series. All of the 7 patients with abdominal wounds who received 15 or more pints of blood for resuscitation died, while only 1 (12.5%) of the 8 extremity wounds receiving similar quantities of blood succumbed. The intermediate position of the group with the combined injuries is probably due to the presence of abdominal injuries of less severity.

Table I. Resuscitation of Battle Casualties - Admission Blood Pressure Imperceptible

Injury	Casualties	Mortality	Receiving 15 Or More Pints of Blood			Receiving Less Than 15 pints of blood		
			No.	No. Died	Mortality	No.	No. Died	Mortality
Abdominal Only	10	70%	7	7	100%	3	0	0
Abdominal and Extremity	6	50%	6	3	50%	0	-	-
Extremity	14	7%	8	1	12.5%	6	0	0
	—	—	—	—	—	—	—	—
Total	30	37%	21	11	52%	9	0	0

Sixty casualties were studied who received 15 - 42 pints of blood (or blood substitute) during the first 24 hours after injury (Table II). Again, a higher mortality is noted in those casualties with abdominal wounds than in those with extremity wounds. Similarly, Table III demonstrates that the incidence of severe renal failure lies predominantly in that group with abdominal wounds.

Table II. Resuscitation Of Battle Casualties - Patients Requiring 15 Or More Pints Of Blood In First 24 Hours

Injury	No.	Total No. Died	Mortality	Number Dying Of Continued Hemorrhage	Mortality Excluding Continuing Hemorrhage
Abdomen	16	13	81%	7	67%
Abdomen and Extremities	20	10	50%	2	42%
Extremities	21	2	9.5%	0	9.5%
Chest	3	2	67%	1	50%
	—	—	—	—	—
Total	60	27	45%	10	34%

Table III. Resuscitation Of Battle Casualties - Patients Requiring 15 Or More Pints Of Blood In First 24 Hours

<u>Injury</u>	No. Living 3 Days Or Longer	Incidence of Renal Failure		
		<u>Anuria</u>	<u>Oliguria</u>	<u>Non-Oliguric Azotemia</u>
Abdomen	9	22%	11%	11%
Abdomen and Extremity	14	28%	0%	7%
Extremity	19	0%	11%	22%
Chest	1	0%	0%	0%
	—	—	—	—
Total	43	14%	7%	14%

Incidence of clinically significant renal failure = 35%.

Mortality in the group which received massive transfusion was due chiefly to continued hemorrhage, to renal failure, and to post-operative hypotension. Two patients with a gangrenous extremity died unexpectedly during the first 48 hours. Pulmonary edema has been clinically so infrequent as to be a rarity.

Type "O" blood has been used throughout this study (24). Crossmatching is not performed. Transfusion reactions have been extremely rare. Blood, in unlimited quantities might not be available, however, to treat casualties in a mass disaster. Preliminary experience in Korea indicates that many of the casualties can be sustained with plasma expanders until evacuation has been achieved. Others, with mild to moderate injuries, can be sustained through surgery, and subsequent evacuation, with plasma expanders. Finally, many of the casualties can be resuscitated with whole blood and plasma expanders in a ratio of one to one. The available plasma expanders leave much to be desired in their present forms. The two under study in Korea, Dextran and modified gelatin, offer promise of moderate support when blood is not available.

To recapitulate, then, the immediate treatment of mass casualties should consist of:

- (1) Control of hemorrhage.
- (2) Correction of serious mechanical defects.
- (3) Movement to a minimal extent - when feasible, transfusion before movement.
- (4) Avoidance of analgesic agents or sedatives whenever possible.
- (5) The early administration of antibiotics.
- (6) The restoration of blood volume. Low titer, group "O" blood can be used without appreciable fear of hemolytic transfusion reaction. Plasma expanders can be used early and can be used as the sole supporting agent during evacuation or operation of those casualties with a blood volume deficit of approximately 1000 - 1500 cc. In wounds of greater magnitude, a ratio of blood to plasma expander of one to one appears promising. Whole blood is, of course, preferable.

Dehydration Survey - This survey was carried out during the period January through July 1952 (25). Objective evidence was sought by selecting volunteers in the

front line and measuring their daily urinary output and its specific gravity. The overall averages for the 265 man days studied was 1,252 cc per day with a specific gravity of 1.022. The lowest urinary output occurred in January, 993 cc. per day, as compared to 1,344 cc. in March, 1,246 cc. in May, and 1,168 cc. in June and July. There was never objective evidence of continued water deprivation.

In a study of 50 casualties on admission to the forward hospital at an average of 3.4 hours after wounding, the average specific gravity was 1.026.

Clinically, dehydration was not noted. Thirst is often an immediate result of wounding and does not signify dehydration at this early stage.

Adrenal Function In The Combat Soldier - The purpose of this study was to survey the combat soldier for evidence of adrenal insufficiency secondary to the chronic stress of battle (26).

For purposes of comparison, a limited study was made of the non-combat troops. The following observations were made:

(1) The eosinophil count was performed daily for 2 or 3 days on 11 soldiers in a non-combat area. The mean count was 142 with a standard deviation of 67.

(2) The average daily excretion of 17 ketosteroids was 13.6 milligrams with a range of 9.9 - 21.9 milligrams. The published normal for the method used is 8.0 - 22.0 milligrams.

(3) The average daily excretion of 11 oxysteroids (formaldehydogenic compounds) was 177 micrograms with a range of 80 - 380 micrograms. The published normal for the method used is 50 - 225 micrograms.

In the front-line soldier the following results were obtained:

(1) The eosinophil count of 17 men obtained daily for 3 days were distributed about a mean of 154 with a standard deviation of 59. Single counts of an additional 21 men were also normal.

(2) The 17 ketosteroid excretion was studied during 68 man days. The results were essentially normal.

(3) Studies of the corticoid excretion in the same group revealed a normal or elevated excretion. The change in excretion from day to day could not be correlated with the day's activities.

No evidence of adrenal exhaustion has therefore been demonstrated.

The Battle Wound - Observations at the surgical hospital and at the evacuation hospital have demonstrated few problems in the management of the battle wound. The policy of debridement and secondary closure still appears to be the safest, most conservative policy. Twenty small wounds were completely debrided and closed primarily. Continued observation revealed normal wound healing. The probability of serious errors in selection, however, prevent the adoption of such a policy. The major problems in wound healing and wound infection has been encountered in the anuric patient. The underlying factors are under study in this group of patients.

The bacterial flora of the war wound has been studied in a selected series of casualties. Cultures of ninety four tissue samples from thirty-three patients have been completed. Ninety-one percent of the patients demonstrated clostridia. Sixty-seven percent of the tissue specimens yielded clostridia. Clostridia sporogenes and Clostridia perfringens were the two species most commonly isolated and were found at all depths of the wound from entrance to exit, in clothing, and in soil. The aerobic flora contained a high incidence of non-hemolytic streptococci, *Proteus*, *Aerobacter*, and

the *Bacillus* species. Hemolytic staphylococci were occasionally found.

Antibiotic sensitivity of 15 species of clostridia from the wounds was determined. Most were sensitive to penicillin, terramycin, and aureomycin, and to a lesser extent to chloromycetin.

Correlation of the changes in the bacterial flora with the progression of wound healing has been undertaken. Results are not yet available.

The Systemic Reaction To Wounding - As mentioned above, a loss of 20-25% of the blood volume is tolerated before the blood pressure drops. A blood loss of 40% (2,000 cc.) is associated with severe shock. Following severe injuries and transfusions the blood pressure can no longer be correlated with blood volume. Massive transfusions may be required to restore the blood pressure. This study of the blood volume is being continued.

Following injury, the red cell shrinks in size. With extremity wounds there is a gradual drop in the hematocrit. With abdominal wounds there is a marked increase in the hematocrit. The hematocrit on admission is of no value in estimating the blood volume deficits.

The white cell count rises rapidly after injury. It is not unusual to note a count of 30,000 to 40,000 on admission. This usually falls during anesthesia. Occasionally, the count falls precipitously toward the end of operation. In these instances, the clinical course of the patient has proved quite serious. The eosinophil count drops to near zero a few hours after injury and then gradually rises over a period of days. A coincident lymphopenia has been described.

Following injury there is an increase in the platelet count and an increase in the fibrinogen concentration of the blood. The prothrombin activity decreases with transfusion due possibly to a deficiency of labile accelerator globulin in the transfused blood. This deficiency may account for the oozing tendency seen after massive transfusion therapy. A few days after injury, the prothrombin activity returns to normal. This is followed a few days later by a secondary drop which is proportionate, both in magnitude and duration, to the magnitude of the original injury.

The anuric patient not infrequently develops a defect in his clotting mechanism. Clinically significant bleeding may occur. Studies of this defect are under way.

With transfusion, the hemolysis of transfused cells produces a rapid rise in the free plasma hemoglobin. If the circulation is adequate, the concentration of hemoglobin rapidly falls. Following shock or abdominal injury, the concentration falls more slowly.

Immediately after transfusion there may be a loss of red cells of considerable magnitude. This is quite marked in some of the patients with very large tissue defects and in those developing post traumatic renal insufficiency.

Changes in renal function are noted following injury. Routinely, one finds a conservation of water and sodium and a loss of potassium for several days. Albuminuria is almost always present and is quantitatively proportional to the magnitude of the injury. Glomerular filtration as measured by inulin clearance, and renal blood flow as measured by para-aminohippurate clearance are markedly reduced following severe injury. Daily clearance studies of endogenous creatinine reveal an elevation in most patients which persists for a period of 7 days.

The patient who is developing post-traumatic renal insufficiency frequently excretes about 500 cc. urine the first day but only 50 cc. the second day. Such a patient, while excreting 500 cc. daily, has a very marked reduction in renal blood flow and in glomerular filtration rate. At this time, however, the ability of the tubules to concentrate the urine is maintained.

Standard hepatic function studies reveal striking abnormalities after injury. The function slowly returns to normal over a period of 7 - 14 days. The following observations were made.

- (1) The serum bilirubin rises sharply during resuscitation and after a few hours begins to fall toward normal. The increment is in the protein bound fraction.
- (2) The urine urobilinogen increases for one or two days after injury and then falls. A second rise occurs about the third to fifth day.
- (3) Bromsulfalein retention is marked after serious injury. The excretion slowly returns to normal over a period of 7 - 14 days.
- (4) The cephalin flocculation test is abnormal and slowly returns to normal.
- (5) Prothrombin activity falls with transfusion. A second fall occurs about the 5th - 8th day.

Studies of adrenal function indicate that the stress response in these patients is of long duration. It would seem probable that the response is the result not of a single trauma but of repeated trauma: Combat stress, anesthesia, operation, secondary debridement, and secondary closures.

Immediately after injury the eosinophil count drops to near zero. There was no exception to this observation after serious injury. Thereafter the count slowly rises and may rise above normal.

The corticoid excretion rises after injury and then returns to normal. A secondary rise may then occur. The excretion is usually invariably proportionate to the eosinophil count. Seventeen ketosteroid excretion frequently rose but not above normal limits. Sodium retention and potassium diuresis are quite marked and may continue for 7 - 14 days.

With injury there is a decrease in salivation and gastric motility. There is a transient decrease in the production of free hydrochloric acid by the stomach. The absorption of water (deuterium oxide) is retarded after injury. After a bowel injury, the absorption of water may be delayed as late as 10 days after injury, even though bowel function appears clinically normal.

Following resuscitation there is a transient rise in plasma hemoglobin and protein bound bilirubin. Albuminuria occurs and others have shown that albumen is lost into the injured tissue. A decrease in the A/G ratio occurs. Fibrinogen increases and prothrombin activity diminishes.

Sodium retention and potassium diuresis follow trauma. Plasma studies of Na, K, Mg, and Ca are under way. Creatine excretion after muscle trauma is quite marked. Creatine excretion may be quite low or exceedingly high.

Sequellae Of Shock and Injury - Post Traumatic Renal Insufficiency: Renal vasoconstriction follows any major injury. As the degree of injury increases, the degree of resulting renal damage increases. It is believed that this renal damage is due primarily to the ischemia. To date, this vasoconstriction cannot be overcome by autonomic blocking agents. As the degree of renal injury increases the clinical course of the patient becomes more critical. Hypertension very often accompanies this renal damage and occasionally may be the most prominent manifestation.

Any seriously injured patient may have a decreased glomerular filtration and renal blood flow as measured by inulin and P.A.H. clearance. Some seriously injured patients, although they maintain a normal urinary output, have a decreased glomerular filtration and a progressive azotemia. Those patients have a stormy clinical course but survive.

The next degree of injury is marked by an oliguria, a more rapid rise in blood protein nitrogen and potassium. If not adequately treated, they may die of this complication. Careful observation and occasional dialysis results in a low mortality.

The most serious stage of renal damage is anuria (less than 100 cc. urine per day). Prior to the development of the Renal Failure Center, the mortality from this complication was approximately 80 to 90% - the patients dying on about the 6th day. The mortality rate is now 50-60% and those patients who die usually do so on the 11th or 12th day.

Abdominal Hemorrhage - This injury may be fatal because of the surgeon's inability to control hemorrhage prior to operation. A therapeutic trial of an intra aortic balloon catheter to control hemorrhage prior to anesthesia in the extreme circumstance has been suggested. As yet no progress has been made on this problem.

Post Operative Hypotension - This syndrome occurs frequently. If untreated, it may lead to a fatal hypotension. It will usually respond to further transfusion even though previous transfusion had been massive. Vasoconstrictors have a limited place in conjunction with transfusion. Observations are now under way on the pressor response to calcium under these circumstances.

Irreversible shock is not recognized prior to anesthesia unless based on continuing hemorrhage or injury to the central nervous system. Shock is sometimes rather refractory to transfusion, however, after massive wounds of the abdomen or extremities. Anesthesia greatly accentuates this refractoriness. Recommendations have been made for continuation of this study both in the field and in the Zone of the Interior.

Gas Gangrene - This complication has been recognized in only about one in a thousand battle casualties. This is in spite of the almost constant contamination of battle wounds with clostridia. Mortality from this complication is now almost zero. The infrequency of this complication is due to early, adequate debridement and to the consistent use of antibiotics.

Vascular Injuries: A study of the treatment of arterial injuries has been a continuing project for the past year (27). The results have clearly demonstrated that primary arterial repair is the treatment of choice. Autogenous vein grafts can be used to bridge larger defects. All arteries of a calibre large enough to permit anastomosis should be repaired. No limitation as to time lapse between injury and repair can be described. The limitation in an individual patient is due to anoxia of the muscle rather than to local arterial changes. Although no limitation can be placed on the time of repair of the individual vessel, the incidence of failure rises sharply after a time lapse of eight hours.

As a result of the project many limbs are being saved in the battle casualties of Korea. The incidence of amputation of an extremity after injury to the popliteal artery has dropped 72% in World War II to approximately 20% in this study.

Hematologic Response to Wounding and to Resuscitation Accomplished by Large Transfusions - Late in 1952, this study was undertaken by Lt. Colonel William Crosby, Army Medical Service Graduate School, and members of the Surgical Research Team in an effort to determine whether or not harmful effects result from massive transfusion of group "O" cells (28).

Thirty-seven casualties were studied at the time of initial resuscitation. Some of these men received 30 or more pints of blood.

The natural results of storing blood - increased plasma hemoglobin and potassium, nonviability of platelets and leukocytes, and low labile factor activity had little or no deleterious effect on patients.

As mentioned earlier, there is a remarkable loss of circulating red cell mass in patients with wounds involving great tissue destruction. This is believed due to hemolysis from an unknown mechanism.

Massive transfusion of group "O" blood into patients of other types resulted in a more or less complete replacement of red cells. These patients at times have plasma antibodies against their own hereditary group. This was observed to produce a gradual hemolysis of native red cells in some cases. No clinical reaction occurred and it is not believed to be a serious matter so long as no effort to made to use a type specific transfusion for at least two weeks.

RENAL INSUFFICIENCY CENTER: This center for therapy of renal insufficiency is in support of the Eighth Army in Korea. While responsibility for the operation was that of the Surgical Research Team, the unit could not have functioned without the wholehearted support of the staff of the 11th Evacuation Hospital and of the Surgeon, Eighth Army.

While renal insufficiency is a relatively infrequent complication of wounding, the mortality from this complication is very high (29). If justification is needed for the extensive and specialized ward-laboratory operation required by these few patients, it lies in the fact that effective therapy is made available to a group of wounded soldiers, increasing each individual's opportunity for survival to an extent not possible before.

After operating the renal insufficiency center for one year the following conclusions are drawn:

(1) Because patients with renal insufficiency present complex problems in physiology and clinical management, maximal efficiency is achieved by concentrating them together with physicians having special training in this field. Likewise, the assigned nurses and medical technicians rapidly learn how to meet the needs peculiar to these patients and become expert in their vital task of continuous care.

(2) The practicality of the Brigham-Kolff type of artificial kidney as a useable instrument in a fixed situation in the field is conclusively demonstrated. Its requirements of 220-volt electric current, pre-heated dialysate bath water, and a flood drain for disposal of used dialysate fluid were easily met. Special arrangements for supply of special non-standard equipment must be made in advance. Additional study will be required before the present instrument can be considered portable enough for close support in a highly mobile type of warfare. It is hoped that these studies may be undertaken in the near future.

(3) This experience demonstrates the value of establishing certain specialized treatment centers within the forward zone of military operations. The unique metabolic behavior of these patients demands prompt and early specialized treatment and renders therapy in areas to the rear relatively less effective. Early helicopter evacuation is well tolerated by these patients. The artificial kidney must always be considered as only an adjunct to good medical care.

(4) Management of patients with renal insufficiency required adequate laboratory support including determination of the major extracellular fluid electrolytes and non-protein nitrogen in blood, urine, vomitus, and gastrointestinal drainage, as well as frequent tracings by a direct-writing electrocardiograph. The latter, together with a flame photometer, manometric Van Slyke apparatus, and accurate analytical methods for other substances, were found to be entirely operable and gave good results under the field conditions experienced.

Summary of Clinical Experiences -

A. Patients treated at the Renal Insufficiency Center:

(1) Post-traumatic renal insufficiency	63
(2) Hemorrhagic Fever	6
(3) Hemoglobinuric nephrosis (transfusion reaction)	2
(4) Burn	1

(5) Early post-wound shock with potassium intoxication	3
(6) Liver failure with subsequent renal insufficiency	1
	—
Total	78

B. Use of the Artificial Kidney, 16 April to 5 October 1952

<u>Disease</u>	<u>No. Patients</u>	<u>No Dialyses</u>
Post-traumatic renal insufficiency	31	72
Hemorrhagic Fever	4	6
Early post-wound shock with potassium intoxication	1	1
Hemoglobinuric nephrosis	3	7
Liver failure with subsequent renal insufficiency	1	1
	—	—
Total	21	87

C. Recovery and mortality statistics in patients with post-traumatic renal insufficiency:

- (1) Prior to arrival of artificial kidney - 1 January to 16 April 1952
 - a. Total number of patients 10
 - b. Total number of deaths 8
 - Overall mortality ... 80%
- (2) After arrival of artificial kidney - 16 April to 31 December 1952
 - a. Total number of patients 55
 - b. Total number of deaths 27
 - Overall mortality ... 51%
 - c. Number of patients dialyzed 31
 - d. Deaths in group c 21
 - Mortality ... 68%

D. Duration of Life in fatal cases:

	<u>No. of patients</u>	<u>Average Day of Death</u>
(1) Before artificial kidney	8	6.2
(2) After artificial kidney; entire group	27	11.1
	Dialyzed group	
	21	12.1

The bulk of the clinical experience involved patients with renal insufficiency following trauma, most of whom were gravely wounded. Their mean evacuation time was three hours from wounding to definitive therapy, usually at Mobile Army Surgical Hospitals. In every instance, available data suggested that these patients had a period of hypotension and had received some low-titer type "O" blood; many received albumin and plasma. The diagnosis of renal insufficiency was uniformly suggested by post-surgical persistence of oliguria and confirmed by water-load tests in some instances.

Hemodialysis achieves only one objective directly: restoration toward normal of disturbed extracellular fluid ionic and molecular concentrations; indirectly, intracellular composition is also affected. Clinically, hemodialysis is followed by

improvement in mental alertness and sense of well-being, relief of nausea and increase of appetite; consequently, bedside care and management proceed more smoothly.

However, the patient's primary surgical problem remains as does the inevitability of chemical and clinical uremic relapse so long as oliguria persists. For this reason, numerical statistics of morbidity and mortality may not be expected to reveal the true value of the technique. Whereas only two patients clearly owe their survival to emergency use of the artificial kidney, it is reasonably certain that most of these patients who expired would have done so much sooner without access to the dialyzer, and usually died when the problems of healing massive wounds, infection, and malnutrition in the presence of prolonged, severe uremia proved overwhelming. Few of the survivors in addition to the two just mentioned would have recovered without hemodialysis because of the severity of their potassium intoxication, and in any event, were aided chemically and symptomatically by the procedure.

The admission procedure should be arranged so that a complete electrocardiogram is available within 5 - 10 minutes of the patient's arrival on the ward, and that emergency therapy, chemical determinations, and indicated immediate surgical therapy may be begun at once and completed within the next one or two hours. Hemodialysis is employed as an adjunct to a regimen of medical management and used whenever indicated. The kidney should be available for the patient upon admission. The regimen for the oliguric phase of acute post-traumatic renal insufficiency consists of the following:

- (1) Restriction of fluid intake (exclusive of blood, plasma, albumin) to equal but not to exceed measured fluid output by urine, vomitus, gastrointestinal output. Obvious sweat losses in febrile patients are compensated for empirically. The volume therapy is guided further by (a) evidence of clinical edema or dehydration, and (b) the daily weight, measured even in unconscious patients on a standard ward scale by means of a rack and litter apparatus of known tare weight.
- (2) Provision of an intake at least of 400 calories (100 grams of glucose) per day and as much more as possible. With restricted volume intake, this necessitates the oral or parenteral use of hypertonic dextrose solutions. The oral route is utilized whenever possible, but drowsiness and vomiting usually terminate this.
- (3) An electrocardiogram daily or oftener to detect potassium intoxication.
- (4) The patient is made to sit up, walk, or move around in bed as much as possible, in an attempt to avoid the pulmonary complications to which these patients are particularly susceptible.
- (5) Potassium ion exchange resins are used orally or per rectum where possible to control the level of plasma potassium.
- (6) Testosterone propionate, along with caloric intake, probably slows the rate of tissue catabolism which in turn is the course (presumably) of the increased NPN and potassium characteristic of this disease.
- (7) Sodium carbonate, calcium gluconate, digitalis, whole blood, packed erythrocytes, sedation, etc. are used judiciously as indicated.

In the diuretic phase, repletion of fluid and electrolytes is based on accurate measurements of the patient's losses, and replacement therapy is designed accordingly. Whereas over-treatment may be the most likely error during the oliguric phase, depletion states from inadequate replacement therapy constitute the major hazard during the diuretic phase.

Clinical Investigation - Effect of Hemodialysis on Cardiovascular Dynamics - Interpretation of the artificial kidney circuit between radial artery and antecubital

vein mimics to a degree an arteriovenous shunt, has been observed to increase arterial and pulse pressure and may improve cardiac function in uremia. A study of these mechanisms was undertaken by measurements of cardiac output and the volume of blood in heart and lungs (central blood volume).

In the majority of instances, pulse rate, pulse pressure, and mean arterial pressure rose and stroke volume fell during dialysis. Cardiac output and peripheral resistance varied reciprocally; the former increased significantly in three patients, decreased in two, and was unchanged in six. In two patients who had pulmonary edema at the start of dialysis, clinical improvement was not associated with changes in central blood volume. No correlation was evident between hemodynamic changes and the variations in blood chemistry caused by dialysis.

The data indicate that dialysis does not create a significant arteriovenous shunt and that the changes it elicits in cardiac output and peripheral resistance are variable.

The Metabolic Response to Trauma and Infection in Patients with Post-Traumatic Renal Insufficiency - A method was devised to estimate rates of accumulation and quantities of new metabolic nitrogen and potassium released during protein catabolism. The increments in plasma concentration were multiplied by the estimated volume of total body water in the case of NPN and by that of extracellular fluid in the case of potassium. The products ascertained could be added algebraically to the respective external balances as customarily determined. Total daily production of those metabolic products could thus be estimated.

The results show that the rates of production of nitrogen and potassium are higher in patients who harbor non-viable or infected tissue than in those who do not. Thus:

<u>Acute Uremia</u>	<u>Average Rate Of New Nitrogen Production</u>
(1) Minor trauma	10 grams/day
(2) Trauma with retained necrotic tissue	20 grams/day
(3) Massive necrosis, generalized peritonitis, severe infection	40-60 grams/day

The rate of accumulation of potassium in extracellular fluid does not correlate with the apparent rate of nitrogen production. This suggests that acute uremia is associated with intracellular accumulation of potassium.

These calculations may serve as estimate of losses of body tissue and the effects thereon of treatment. They demonstrate the large metabolic differences imposed by different degrees of wounding and infection. They measure the rate of protein catabolism; this is in comparison with weight loss indicating the rate of breakdown of body fat and of liberation of body water.

Intracellular Sodium and Potassium - The amount of potassium removed during dialysis into the bath is always greater than the decrement of potassium in extracellular fluid. It follows that potassium has therefore been removed from the cells. An attempt was made to estimate changes in cell contents from the changes in sodium and potassium concentrations in erythrocytes, since it seemed that these cells might participate in this shift of potassium.

The analyses depend on determinations of sodium and potassium in whole blood and plasma and estimations of cell content by reference to the hematocrit. In nine normal individuals erythrocyte concentrations of sodium averaged 11.9 meq/L and of potassium 97.7 meq/L. In patients before dialysis erythrocyte sodium was usually increased and potassium normal; these pre-dialysis levels could not be correlated with estimates of cell concentration from external balance or previous therapy.

SPECIAL INVESTIGATION OF BLOOD USAGE AND BLOOD STORAGE: During 1952, a continuing effort was made to establish an efficient blood banking and blood distribution program. As in previous years, most of the blood was obtained from the Zone of the Interior and was supplemented by blood drawn in Japan. The donor panel available in Japan was primarily intended to meet emergency requirements in Korea, and to provide blood of types other than "O" for use in hospitals in Japan. According to operating plan, it was not intended that the blood locally drawn would reduce materially the amount of blood routinely obtained from the United States. Since blood was sent to Korea whenever requested, it was felt advisable to survey blood bank, blood distribution, and blood use policies in Korea. The survey, fortunately, coincided with a period of considerable enemy activity. As a result of this survey, and a study of blood receipts and issue data for the entire year, it became apparent that under the military situation in Korea a more organized program might be formulated. There are two points to be attacked: providing the freshest blood possible, and decreasing the amount of blood kept in reserve by means of a more efficient distribution program. These problems are easily overcome.

During 1952, approximately 100,000 bottles of blood were received from the United States, and 35,000 bottles were collected in Japan. During the same period, approximately 85,000 bottles of blood were sent to Eighth Army, 30,000 to KCOMZ, and 20,000 to hospitals in Japan. It was not possible to be certain of the number of bottles issued to Eighth Army per wounded in action because this blood was made available to a significant but unknown percentage of Koreans who were wounded in action. Approximately 2 bottles of blood per wounded in action was issued to Eighth Army if all Korean wounded are included, and 4.75 bottles per wounded in action if only U.S. and UN other than Koreans are considered.

During 1952, reserve blood depots were maintained in Tokyo, Pusan, and Seoul, and three advance subdepots in the Eighth Army area. In addition, each hospital maintained a reserve to meet emergencies. Although there was no actual loss of blood through wastage, the multiple reserve levels resulted in blood aging in the depots. This was recognized early in 1952, and the policy of sending the freshest blood possible to the hospitals was emphasized. The reserve level during the survey period in 1952 was approximately three times the maximum used during any one day. Thus there was a three day supply on hand. Since approximately three days elapse between the time emergency requests are sent to the ZI and blood is received from the ZI, this would appear to be a minimum level that could be safely maintained. However, this survey occurred during a period of heavy activity. In less active periods, the reserve level represented almost an 8 day supply. This reserve level appears to be excessive.

During the survey it was found that approximately 20% of the wounded in action were transfused, receiving an average of 4.3 bottles of blood per patient, or 0.9 bottles per wounded in action. This experience indicated much more liberal use of blood than was reported during World War II and there was some indication that the trend toward the use of still larger amounts of blood in the treatment of wounding is continuing.

WOUND BALLISTICS AND BODY ARMOR: The medical service personnel of the Wound Ballistics Team were based on the Far East Medical Research Unit during their visits to the Far East Command in 1952. Body Armor investigation was a combined effort of the Medical Service and the Quartermaster Corps.

The findings of these teams cannot be published at this time due to their classification. It can be stated, however, that this is an important and continuing field of medical research.

NEUROPSYCHIATRIC RESEARCH TEAM: This team was still in the earliest stages at the close of 1952 with only one project actually in progress. Captain Roger W. Little undertook a study of the effect of the social system of a combat unit upon stress and combat fatigue. The study is in progress but only preliminary reports are available at this time.

PUBLICATIONS AND MANUSCRIPTS

Department of Entomology

Articles Published:

- (1) Parasitic Mites Found on Small Mammals in Japan and Korea, Anonymous. Office of the Chief Surgeon, Far East Command, 14 pages, 10 plates of figures, March 1952.
- (2) The Fleas of Japan and Korea, Anonymous. Office of the Chief Surgeon, Far East Command, 22 pages, 26 plates of figures, January 1953.
- (3) On the Occurrence of the Parasitic Mite, Haemogamasus quadrisetatus Vitzthum, 1926 (Acari: Laelaptidae) in Japan, Asanuma, Kiyoshi, E. W. Jameson, Jr., and Kayoko Sekikawa. Misc. Reports Research Inst. for Nat. Resources No. 26: 71-76, 1952.

Articles Submitted for Publication:

- (4) A New Species of Biting Midge (Ceratopogonidae) from Japan, Masaaki Tokunaga and Yukio Shogaki.
- (5) Shunsennia tarsalis, a New Genus and Species of Chigger from Korea (Acarina: Trombiculidae), E. W. Jameson, Jr. and Seiichi Toshioka.
- (6) Trombicula (Trombiculindus) kansai, A New Chigger from Central Honshu, Japan. E. W. Jameson, Jr. and Manabu Sasa.
- (7) Two New Hystrichopsyllid Fleas from Japan (Siphonaptera: Hystrichopsyllidae). Dr. E. W. Jameson, Jr. and Nobuo Kumada.

Department of Medical Zoology

Articles Published:

- (1) Parasitological Studies in the Far East. V. An Epidemiologic Survey of Okayama Prefecture, Honshu, Japan, Ritchie, L. S., Hunter, G. W. III, Kaufman, E. H. Jr., Pan, C., Nagano, K., Yokogawa, M. and Szewczak, J. T. Jap. Med. Jour. 4(5): 307-314, 1951.
- (2) Studies on Schistosomiasis. V. Testing Protective Ointments Against Schistosomiasis by Using Schistosome Dermatitis Producing Cercariae, Hunter, G. W. III, Ritchie, L. S., Tanabe, H., Nagano, K., Pan, C. and Yokogawa, M. Trans. Roy. Soc. Trop. Med. and Hyg., 46(2): 201-206, 1952.
- (3) A Comparison of the Infectivity of Schistosoma japonicum Occurring in Japan for Oncomelania nosophora and Oncomelania formosana, Hunter, G. W. III, Ritchie, L. S. and Otori, Y. J. Parasit., 38(5): 492, 1952.
- (4) Studies on Schistosomiasis. VI. Control of the Snail Host of Schistosomiasis in Japan with Sodium Pentachlorophenate (Santobrite), Hunter, G. W. III, Freytag, R. E., Ritchie, L. S., Pan, C., Yokogawa, M. and Potts, D. E. Am. J. Trop. Med. and Hyg., 1(5): 831-847, 1952.
- (5) A Comparison of the Zinc Sulfate and the MGL (Formalin-Ether) Technics. Ritchie, L. S., Pan, C. and Hunter, G. W. III. J. Parasit., 38 (Suppl.): 16 (Abstract), 1952.
- (6) Efficiency of the Formalin-Ether Concentration Technic, Wykoff, D. E. and Ritchie, L. S. J. Parasit., 38 (Suppl.): 15 (Abstract), 1952.

- (7) Studies on Schistosomiasis. IV. Two Ointments For Protection Against Schistosomiasis japonica, Ritchie, L.S., Hunter, G.W. III, Nagano, K. and Lin, S. Mil. Surg., Vol. III, No. 2, August, 1952.
- (8) Potential Molluscacides Screened in the Laboratory and the Results of Preliminary Field Plot Tests, Hunter, G. W. III, Freytag, R. E. and Ritchie, L. S. J. Parasit. 38(6): 509-516, 1952.

Articles Submitted for Publication:

- (9) The Distribution of the Snail Intermediate Host of Schistosoma japonicum (Oncomelania nosophora) Along the Tone River, Japan, Ritchie, L. A., Hunter, G.W. III, Nagano, K. and Pan, C. Am. Jour. Trop. Med. and Hyg.
- (10) Parasitological Studies in the Far East. VI. An Epidemiologic Survey of the Tone River Area, Japan, Ritchie, L.S., Hunter, G. W. III, Pan, C., Yokogawa, M. and Szewczak, J. T. Jap. Med. Jour.
- (11) Parasitological Studies in the Far East. VIII. An Epidemiologic Survey of the Tone River Area, Japan, Ritchie, L.S., Hunter, G. W. III, Pan, C., Yokogawa, M., Nagano, K. and Szewczak, J. T. Jap. Med. Jour.
- (12) Parasitological Studies in the Far East. IX. An Epidemiologic Survey in Shikoku Island, Japan, Hunter, G.W. III, Ritchie, L.S., Pan, C., Yokogawa, M. and Altamirano, M. Jap. Med Jour.
- (13) Parasitological Studies in the Far East. I. Methods and Review of Japanese Literature, Tigertt, W.D., Hunter, G.W. III, and Ritchie, L.S. Jap. Med. Jour.
- (14) Reinfection and Seasonal Fluctuations of Ascaris lumbricoides, Pan, C., Ritchie, L.S. and Hunter, G. W. III.
- (15) The Penetration Time for the Cercariae of Schistosoma japonicum, Pan, C., Williams, R.R. and Ritchie, L.S.

DEPARTMENT OF PATHOLOGY

Articles Published:

- (1) Pathology of Hemorrhagic Fever, Steer, A. and Hüllinghorst, R.L., Year Book of Pathology, 1952.
- (2) Pathology of Hemorrhagic Fever, Hüllinghorst, R. L. and Steer, A., Ann. Int. Med, 53: 77-101, January, 1953.
- (3) Experience with Needle Liver Biopsies of the Hepatitis Center for Japan and Korea, 1950-1951, Deschamps, S.H. and Steer, A. Am. J. Med., 13: 674-688, December 1952.
- (4) Plastic Bags for Shipment of Tissue Specimens, Steer, A. U. S. Armed Forces Medical Journal, 4: 617-619, April 1953.

DEPARTMENT OF SEROLOGY

Articles Published:

- (1) Rh and ABO Blood Group Distributions in Japanese and Ethiopians, Stein, G. J., Hilton, K. C. and Mason, R. P. Science, 117: 100-101, January, 1952.

FAR EAST MEDICAL RESEARCH UNIT

Articles Published:

- (1) The Bacteriologic Diagnosis of Enteric Infections, Hardy, A. V., Mason, R. P., Hamerick, D., Mitchell, R. B. U. S. Armed Forces Medical Journal, 4:541-553, April 1953.
- (2) The Dysenteries in the Armed Forces, Hardy, A. V., Mason, R. P. and Martin, G. A. Am. Jour. of Trop. Med. and Hyg., 1:171-175, January, 1952.
- (3) Antibiotics in Acute Bacillary Dysentery: Observations in 1408 Cases with Positive Cultures, Garfinkel, B. T., Martin, G. A., Watt, J., Payne, F. J., Mason, R. P. and Hardy, A. V. J.A.M.A., 151: 1157-1159, April 4, 1953.

REFERENCES

Department of Epidemiology

1. Mayer, Claudius, F., Epidemic Hemorrhagic Fever, Preliminary Draft. Washington, D.C., December 1951.
2. Smorodintsev, A.A., et al, Etiologiya Gemorragicheskogo Nefroso-nephritis. Moscow Medgiz, 1944.
3. Annual Report, 406th Medical General Laboratory, 1950.
4. Annual Report, 406th Medical General Laboratory, 1951.
5. Personal Communication, Statistical Division, Ministry of Health of Japan.
6. Rivers, Thomas M., Viral and Rickettsial Infections of Man, 4th Ed., J. B. Lippencott Co., Philadelphia, 1948.

Department of Virus and Rickettsial Diseases

1. Paul, J.R.: Laboratory Methods of the United States Army. Lea and Febiger, 1944, pp 579-600.
2. Hammon, W. McD.: Diagnostic Procedures for Virus and Rickettsial Diseases, Am. Pub. Health Assn., 1st Ed, 1948, pp 187-218.
3. Annual Report, 406th Medical General Laboratory, 1950, Pathology Section, p 145.
4. Belson, P.B. and Hankey, D. D.: Benign Aseptic Meningitis as a Manifestation of Leptospiral Infection. Tr. S. Am. Physicians. 63, 130, 1950.
5. Gauld R. L., Crouch, W.L., Kaminsky, A. L., Hullinghorst, R. L., Gochenour, W. S., Yager, R.H.: Leptospiral Meningitis: Report of Outbreak Among American Troops on Okinawa, J. Am. Med. Assn., 149, pp 228-231, 1952.
6. Curnen, E. C., Shaw, E. W., and Melnick, J. L.: Disease Resembling Non-paralytic Poliomyelitis Associated with a Virus Pathogenic for Infant Mice. J. Am. Med. Assn., 141, pp 894-901, 1949.
7. a. von Magnus, H.: Isolering af tre virus stammer af Coxsackie-Gruppen fra patienter med Meningeale Symptomer. Ugeskrift for Laeger, 111, 1451-1453, 1949.
b. Gard, S.: "C Virus" som orsak till "barnforlamning" uton pareser. Svenska Lakartidningen, 47, pp 285-290, 1950.
8. Hilleman, M.: System for Measuring and Designating Antigenic Components of Influenza Viruses with Analyses of Recently Isolated Strains. Proc. Soc. Exp. Biol. and Med., 1951, 78, pp 208-215.
9. Mitamura, T., Kitaoka, M., Mori, K., and Kobayashi, E.: Evidence of Inapparent Infection with Japanese B Encephalitis Virus in Healthy Looking Persons and Animals. Tokyo Ijishinshi, 62, 779-789, 1938 (in Japanese).
10. a. Smorodintsev, A.A.: J. Mikrobiol., Epidemiol., Immunobiol., Moskva, 1942, pp 11-12, 67.
b. Schublade, A. L.: J. Mikrobiol., Epidemiol., Immunobiol., 1943, pp 1-2, 87, as reviewed by Rosental, L. Am. Review of Sov. Med., 1944, 2, p 166.

11. Hammon, W. McD., Reeves, W. C., and Burroughs, R.: Japanese B Encephalitis Virus in the Blood of Experimentally Inoculated Chickens. *Proc. Soc. Exp. Biol. and Med.*, 1946, 61: 304-308.
12. Hammon, W. McD. and McClure, H. E.: Quoted from Annual Report, 406th Medical General Laboratory, 1951, p 275.
13. Hammon, W. McD., Reeves, W. C., and Sather, G.E.: Japanese B Encephalitis Virus in the Blood of Experimentally Inoculated Birds. *Am. J. Hygiene*, 1951, 53, p 249.
14. Sabin, A. B.: Epidemic Encephalitis in Military Personnel: Isolation of JBE Virus on Okinawa in 1945, Serologic Diagnosis, Clinical Manifestations, Epidemiologic Aspects and Use of Mouse Brain Vaccine. *J.A.M.A.*, 1947, 133, p 281.
15. Bawell, M. B., Deuel, R. E., Jr., Matumoto, M., and Sabin, A.B.: Status and Significance of Inapparent Infection with Virus of Japanese B Encephalitis in Japan in 1946. *Am. J. Hygiene*, 1950, 51, 1.
16. Deuel, R. E., Jr., Bawell, M. B., Matumoto, M., and Sabin, A.: Status and Significance of Inapparent Infection with Virus of Japanese B Encephalitis in Korea and Okinawa in 1946. *Am. J. Hygiene*, 1950, 51, 13.
17. Barnett, H. C.: Quoted from Annual Report, 406th Medical General Laboratory, 1950, p 196, 200, and 1951, p 233.
18. Mitamura, T., Mori, K., Kitaoka, M. and Tenjin, S.: Tokyo Iji Shinshi, 1947, 3076, pp 812-819. (In Japanese).
19. Kobayashi, R., Ando, K., Toyama, Y., Kuratsuka, K., Arima, S., Saito, K., Kakayama, Y., Hironaka, N., Ishii, K., Honda, Y., and Konde, K.: On Susceptibility of Japanese Wild Birds for Japanese B Encephalitis. *Jap. Med. Jour.*, 1948, 1:282-288.
20. Itoh, H., Toda, M., Shimada, F., and Fukuzumi, S.: Experimental Studies on the Virus of Japanese B Encephalitis Using Chickens. Development and Persistence of Serum Antibodies. *Kitasato Arch. of Exp. Med.*, 1951, 24:67-75.
21. Hammon, W. McD., Rees, D. M., Casals, J., and Meiklejohn, G.: Experimental Transmission of Japanese B Encephalitis Virus by Culex tritaeniorhynchus and Culex pipiens var pallens, Suspected Natural Vectors. *Am. J. Hyg.*, 1949, 50:46-50.
22. Annual Report, 406th Medical General Laboratory, Professional Section, 1949, p 149.
23. Sabin, A. B. and Buescher, E. L.: Unique Physico-Chemical Properties of Japanese B Encephalitis Virus Hemagglutinin. *Proc. Soc. Exp. Biol. and Med.*, 1950, 74, pp 222-230.
24. Buescher, E. L.: Quoted from Rivers' *Viral and Rickettsial Infections of Man*, 1952, 2nd Ed, p 86.
25. Anonymous, 1951: Rickettsiae and Viruses, in *Methods for Medical Laboratory Technicians*, Departments of the Army and the Air Force, TM 8-227, AFM 160-14, Washington, U. S. Government Printing Office, pp 523-558.
26. Annual Report, 406th Medical General Laboratory, 1950, p 211.
27. Hirst, G. L.: The Nature of Virus Receptors of Red Cells I. Evidence of the Chemical Nature of the Virus Receptors of Red Cells and of the Existence of a Closely Analogous Substance in Normal Serum. *J. Exp. Med.*, 1948, 87, p 41.

28. Jordan, J. and Mirick, G. S.: Hepatitis in Mice of Presumed Viral Origin, A Preliminary Report. Bull. Johns Hopkins Hosp, 1951, 89, 326.

29. Gledhill, A. W. and Andrewes, C. H.: A Hepatitis Virus of Mice. Brit. J. Exp. Path., 1951, pp 559-568.

30. Nelson, J. B.: Acute Hepatitis Associated with Mouse Leukemia. I. Pathological Features, and Transmission of the Disease. J. Exp. Med., 1952, 96, 4 pp 293-302.

31. Nelson, J. B.: Acute Hepatitis Associated with Mouse Leukemia. II. Etiology and Host Range of the Causal Agent in Mice. J. Exp. Med., 1952, 96, 4, pp 302-312.

32. Smorodintsev, A. A., Al'shuler, I. S., Dunaevskii, M. I., Kokhreidze, K. A., Neustroev, V. D. and Churilov, A. V.: Etiologiya Gemorragicheskogo Nefroso-nephritis. Moscow Medgiz, 1944, pp 28-38.

Department of Entomology

1. Lipovsky, Louis J., A Washing Method of Ectoparasite Recovery with Particular Reference to Chiggers (Acarina-Trombiculidae). Jour. Kansas Ent. Soc. 24(4):151-156, 1951.

2. Lipovsky, Louis J., Personal Communications, 1952.

3. Wharton, G. W. and H. S. Fuller, A Manual of the Chiggers. Mem. Ent. Soc. Washington No. 4 185, pp, 1952.

4. Nagayo, M., Miyagawa, Y., Mitamura, T., Tamiya, T., and Tenjin, S. Five Species of Tsutsugamushi (the Carrier of Japanese River Fever) and Their Relation to the Tsutsugamushi Disease. Amer. Jour. Hyg., 1:569-591, 1921.

5. Kawamura, R., Studies on Tsutsugamushi Disease (Japanese Flood Fever). Med Bull. College Med. Univ. Cincinnati 4 (1 and 2):ix / 229 pp, illus. 1926.

6. Fukuzumi, Sadakichi, Obata, Y., and Kagiwada, R. On the Trombiculid Mite and Rickettsia Discovered in the Mount Fuji Foot Plain. Kitasato Arch. Exp. Med. 23(4): 12-14, 1951.

7. Annual Report, 406th Medical General Laboratory, 1948.

8. Shiraki, Tokuchi, Simuliidae of the Japanese Empire. Mem. Faculty Sci. Agric., Taihoku Imperial Univ., 16(1):1-90, 14 pl., 1935.

9. Kano, Horomichi, and Takahashi, H., Die Simuliiden von Sachalin und Hokkaido. Ins. Matsumurana 14(2 and 3):79-82, 1940.

10. Takahashi, Hiroshi, Description of a New Species of Simuliidae from Nippon, with other notes. Ins. Matsumurana 15(3):86-88, 1941.

11. Smart, J., The British Simuliidae, with Keys to the Species in the Adult, Pupal and Larval Stages. Freshwater Biological Association of the British Empire, Sci. Publ. No. 9, 57 pp., 1944.

12. Barnett, H. C., and Toshioka, S., The Blood Sucking Insects, Mites, and Ticks of Korea, and Their Relation to Disease Transmission. 406th Medical General Laboratory, 25 pp., 1951.

13. Parasitic mites Found on Small Mammals in Japan and Korea. Office of the Chief Surgeon, Far East Command, 14 pp., 10 plates of figures, March 1952.

14. The Fleas of Japan and Korea. Office of the Chief Surgeon, AFPE, 22 pp., 26 plates of figures, January 1953.
15. Department of Entomology, 406th Medical General Laboratory, Annual Report for 1951.
16. LaCasse, Walter J., and Satyu Yamaguti, Mosquito Fauna of Japan and Korea. Office of the Surgeon, Hq. Eighth Army, Revised Ed., 481 pp., 1950.
17. Sasa, M. and Sabin, A.B., Ecological Studies on the Mosquitoes of Okayama in Relation to the Epidemiology of Japanese B Encephalitis. Amer. Jour. Hyg. 51(1): 21-35, 1950.
18. Department of Entomology, 406th Medical General Laboratory, Annual Report for 1950.
19. Mitamura, T., Mori, K., Kitaoka, M., and Tenjin, S., Experimental Transmission of Japanese "B" Virus by Artificially Infected Mosquitoes. Tokyo Iji Shinshi, 3076:812-819, 1947.
20. Hammon, W. McD., Rees, D., Casals, J., and Meiklejohn, G., Experimental Transmission of Japanese "B" Encephalitis Virus by Culex tritaeniorhynchus and Culex pipiens var. pallens suspected natural vectors. Amer. Jour. of Hyg. 50(1):46-50, 1949.
21. Annual Report, 406th Medical General Laboratory, 1949.
22. Reeves, W.C., and Hammon, W. McD., Laboratory Transmission of Japanese "B" Encephalitis Virus by Seven Species (Three Genera of North American Mosquitoes). Jour. of Exp. Med. 83:185-194, 1946.
23. Trembly, H. L., Mosquito Culture Technique. Mosquito News, 4:103-119, 1944.
24. Mitamura, T., and Kitaoka, M., Reports of the 11th Meeting of the Encephalitis Committee. a. On the Transovarian Infection of Mosquitoes. Tokyo Iji Shinshi, 63:1884-1886, (In Japanese), 1939.
25. Mitamura, T., et al, Experiments on the Transmission of the Virus by Various Species of Mosquitoes Artificially Infected with the Virus of Japanese Epidemic Encephalitis. Tokyo Iji Shinshi, 62:812-819, (In Japanese), 1938.
26. Scott, J. S., Stembridge, V.A., and Sisley, N. M., A Method for Providing a Constant Supply of Tropical Rat Mites, etc., Jour. of Parasit., 33(2):138-141, 1947.
27. Hein, S. A., Technical Experience in the Breeding of Tenebrio molitor, Karinklijke Akademie van Wetenschappen to Amsterdam 23(1):192-218.
28. Linduska, J.P. and Cochran, J.H., A Laboratory Method of Flea Culture. Jour. of Econ. Ent., 39(4):544-5, 1946.
29. Personal Communication, U.S. Department of Agriculture Laboratory, Orlando, Florida to Lt. Colonel Robert Traub, 1952.
30. Hollenbeck, A.H., A Practical Method for Mass Production and Transfer of Xenopsylla cheopis. Jour. of Parasit. 32(5):463-4, 1946.

Department of Chemistry

1. Baird Associates Flame Photometer Manual, Baird Associates, 33 University Road, Cambridge 38, Massachusetts.
2. Daughaday, W.H., Jaffe H., and Williams, R.H. Chemical Assay of Cortin; Determination of Formaldehyde Liberated on Oxidation with Periodic Acid. J. Clin. Endoc., 8:166, 1948.
3. Somogyi, M.: Micromethods for the Estimation of Diastase. J. Biol. Chem., 124:179, 1938.
4. Folin, O. and Wu, H.: Determination of Glucose in Blood. J. Biol. Chem., 38:106, 1919.
5. Shriner, R.L. and Fuson, R.C.: Identification of Organic Compounds, 2d Ed. John Wiley and Sons, Inc., London, 1940, p. 138.
6. Gradwohl, R.B.H.: Clinical Laboratory Methods and Diagnosis, 4th Ed., Vol. II. The C.V. Mosby Co., St. Louis, Mo., 1948, p. 2042.
7. Gross, E.G. and Thompson, F.: The Excretion of a Combined Form of Morphine in Tolerant and Non-tolerant Dogs. J. Pharm. and Exper. Therap., 68:413, 1940.
8. Goldbaum, L.R.: An Ultraviolet Spectrophotometric Procedure for the Determination of Barbiturates. J. of Pharm. and Exper. Therap., 94:68, 1948.
9. Annual Historical Report, 406th Medical General Laboratory, 1950, pp. 111-113.
10. Hynds, C.E.: Report of Horse Meat in Ground Meat. J.A.O.A.C., 33:752-753, 1950 and 34:355-357, 1951.
11. Annual Historical Report, 406th Medical General Laboratory, 1951, p. 176.
12. Roe, J.H., Mills, M.E., Oesterling, M.J., and Dameron, C.M.: The Determination of Diketo-l-gulonic Acid, Dehydro-l-ascorbic Acid, and l-ascorbic Acid in the Same Tissue Extract by the 2,4 Dinitro-phenylhydrazine Method. J. Biol. Chem., 174:201-208, 1948.
13. Pepkowitz, L.P.: The Rapid Determination of Ascorbic Acid by the Adaptation of Stotz's Method to Plant Materials. J. Biol. Chem., 151:405.
14. Hawk, P.B., Oser, B.L., and Summerson, W.H.: Practical Physiological Chemistry. The Blakiston Company, Philadelphia, Pa., p. 1066.
15. Winton, A.L., and Winton, K.E.: The Analysis of Foods. John Wiley and Sons, Inc., p. 338.
16. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 7th Ed. Association of Official Agricultural Chemists, Washington, D.C., par. 40.32, 40.33, 40.34, 40.35.
17. Annual Historical Report, 406th Medical General Laboratory, 1951, pp. 183-194.
18. Schrenk, H.H., Heinmann, H., Clayton, G.D.: Gaffer, W.M., Wexler, H.: Air Pollution in Donora, Pennsylvania. Public Health Bulletin No. 306. Federal Security Agency, Division of Industrial Hygiene, Washington, D.C., 1949.
19. Second Technical and Administrative Report of Air Pollution Control in Los Angeles 1950-1951. Air Pollution Control District, County of Los Angeles.
20. Ibid reference 19.

21. Jacobs, M.B.: Analytical Chemistry of Industrial Poisons, Hazards, and Solvents. Interscience Publishers Inc., New York, N.Y., 1944.
22. Ibid reference 21, pp. 245-247; 279-287.
23. Air Pollution, A Symposium. Industrial and Engineering Chemistry, June, 1952. pp. 1339-1380.
24. Ibid reference 19.
25. Eisenbud, M. and Harris, W.B.: Meteorological Technics in Air Pollution Surveys, A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 3, No. 1, pp. 90-97, 1951.
26. Mills, C.A.: Nontuberculous Diseases of the Chest and Related Matters, Implications of Air Pollution in Chronic Respiratory and Cardiac Disorders. Transactions of the Forty-Seventh Annual Meeting of the National Tuberculosis Association, 1951.
27. Ibid reference 18.
28. Mills, C.A.: Excess Respiratory and Cardiac Deaths in Los Angeles Smogs. 109th National Meeting of American Meteorological Society, 1951.
29. Standard Methods for the Examination of Water and Sewage. American Public Health Association, New York, N.Y., 8th Edition.
30. Ibid reference 29, p. 140.
31. Babbitt, H.E. and Doland, J.J.: Water Supply Engineering. McGraw-Hill Book Company, Inc., New York City, N.Y., 3d Edition, 1939.
32. Annual Historical Report, 406th Medical General Laboratory, 1951, pp. 178-183.
33. Officers of the United States Pharmacopoeial Convention 1940-1950: Pharmacopoeia of the United States, Thirteenth Revision, 1947., p. 600.
34. The United States Pharmacopoeial Convention, Inc.: The Pharmacopoeia of the United States, Fourteenth Revision, 1950.
35. Osol, A. and Farrar, G.E.: The Dispensary of the United States of America, 24th Edition.
36. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 7th Edition. Association of Official Agricultural Chemists, Washington, D.C.
37. Ibid reference 32, p. 198-202.
38. Holtorff, A.F. and Koch, F.C.: The Colorimetric Estimation of 17-Ketosteroids and Their Application to Urine Extracts. J. Biol. Chem., 135:377-392 (Sept 1940).
39. Lubschez, R.: J. Biol. Chem., 183:731, 1950.
40. Code, C.F.: The Quantitative Estimation of Histamine in the Blood. Am. J. Physiol., 127:78-93, Aug. 1939.
41. Scudder, J.: Blood Substitutes and Blood Transfusions, Edited by Stuart Mudd and Wm. Thalheimer, C.C. Thomas, 1942.
42. Reiner, M. and Fenichel, R.L.: Dialysis of Protein Solutions for Electrophoresis. Science, 108:164, 1948.

43. Stern, K. G. and Reiner, M.: Electrophoresis in Medicine. Yale J. Biol. and Med., 19:67, 1946.
44. Ibid reference 43.
45. Annual Historical Report, 406th Medical General Laboratory, 1951, p. 165.
46. Parpart, A.K., Lorenz, P.B., Parpart, E.R., Gregg, J.R., and Chase, A.M.: The Osmotic Resistance (Fragility) of Human Red Cells. J. Clin. Invest., 26:636-640, 1947.
47. Sack, T., Gibson, J.G., and Buckley, E.S.: The Preservation of Whole ACD Blood Collected, Stored, and Transfused in Plastic Equipment. Surgery, Gynecology, and Obstetrics, 95:113-119., July 1952.
48. Block, R.J., LaStrange, R., and Zweig, G.: Paper Chromatography, A Laboratory Manual. Academic Press Inc., New York City, N.Y., 1952, p. 4.
49. Consden, R., Gordon, A.H., and Martin, A.J.P.: Biochem. J., 38:230, 1944.
50. Gross, E.G. and Thompson, V.J.: The Excretion of the Combined Form of Morphine in Tolerant and Non-tolerant Dogs. J. Pharm. and Exper. Therap., 68:413, 1940.
51. Adler, T.K. and Shaw, F.H.: The Biological Liberation of Morphine from Codeine in the Rat. J. Pharm. and Exper. Therap. 104:1-10, 1952.
52. Pierce, I.H. and Plant, O.H.J.: Studies of Chronic Morphine Poisoning in Dogs IV. Excretion of Morphine in Tolerant and Non-Tolerant Animals. J. Pharm. and Exper. Therap., 46:201, 1932.
53. Zauder, H.L.: Effect of Prolonged Morphine Administration On the In Vivo and In Vitro Conjugation of Morphine in Rats. J. Pharmacol. and Exper. Therap., 104:11-19, 1952.

Department of Bacteriology

1. DA TM 8-227: Methods for Medical Laboratory Technicians, August, 1951.
2. Teague, O., and Diebert, O: The Value of the Cultural Method in the Diagnosis of Chancroid, Jour. Urol. 4: 543-550, 1948.
3. Moore, J.E.: The Diagnosis of Chancroid and the Effect of Prophylaxis Upon Its Incidence in the American Expeditionary Forces, Jour. Urol. 4: 1920.
4. Greenblatt, R.B., and Sanderson, E.S.: The Intradermal Chancroid Bacillary Antigen Test as an Aid in the Diagnosis of the Venereal Bubo, Am. J. Surgery, 91: 384-392, 1938.
5. Beeson, P.B., and Heyman, A.: Studies on Chancroid: Efficiency of the Cultural Method of Diagnosis, Am. Jour. Syph., Gon., and Vener. Dis. 29: 633-40, 1945.
6. Annual Historical Report, 406th Med. Gen. Lab, 1950: Bacteriology Section.
7. Annual Historical Report, 406th Med. Gen. Lab., 1951: Bacteriology Section.
8. Coutts, W.E.: Non-bacterial Infection of the Urinary Tract, Brit. J. Ven. Dis., 24: 109-20, 1948.
9. Gorman, E., and Liebowitz, A.: Non-gonococcal Urethritis Preliminary Report, Med. Bull. US Army FE. 1: 4-7, 1952.

10. Baier, G.F.: Non-specific Urethritis, Bull, US Army Med Dept., 2: 679-81, 1949.
11. Spink, W.W.: Clinical and Biological Significance of Penicillin-Resistant Staphylococci, Including Observations with Streptomycin, Aureomycin, Chloramphenicol, and Terramycin, J. Lab. Clin. Med., 37: 278-298, 1951.
12. Bondi, A. and Dietz, C.C.: Penicillin-Resistant Staphylococci, Proc. Soc. Exper. Biol. Med., 60: 55, 1945.
13. Barber, M.: Coagulase-Positive Staphylococci Resistant to Penicillin, J. Path. Bact., 59: 373-384, 1947.
14. Eagle, H. and Musselman, A.D.: The Rate of Bactericidal Action of Penicillin in vitro as a Function of its Concentration and its Paradoxically Reduced Activity for Certain Organisms, J. Exp. Med., 88: 99-131, 1948.
15. Gudgel, E.F., Major, MC: Personal Communication, 1952.
16. Lewis, G.M., and Hopper, M.E.,: Introduction to Medical Mycology. Year-book, 1948.
17. Sherman, J.M.: The Streptococci, Bact. Rev., 1: 3-97, 1937.
18. Edwards, P.R.: The Biochemical Characters of Human and Animal Strains of Hemolytic Streptococci, J. Bact. 23: 259-266, 1932.
19. Altemeier, W.A.: Bacteriology of War Wounds, Internat. Abstracts Surg., 75: 518-533, 1942.
20. Altemeier, W.A.: Bacteriology of Traumatic Wounds, J.A.M.A., 124: 143-147, 1944.
21. Smith, L. de S.: Clostridia in Gas Gangrene, Bact. Rev. 13: 233-254, 1949.
22. Stock, A.H.: Anaerobic Spore-bearing Flora of Gas Gangrene, Italy Med. Bull, Mediterranean Theater of Operations, 2: 159-162, 1944.
23. Rustigian, R., and Cipriani, A.: The Bacteriology of Open Wounds, J.A.M.A., 133: 224-230, 1947.
24. MacLennan, J.D.: Anaerobic Infections in War Wounds of the Middle East, Lancet., 2: 63-66, 94-99, 123-126, 1943.
25. Howard, John, Captain, MC, Personal Communication, 1952.
26. Weinburg, M., and Seguin, P.: La Gangrene Gazeuze, Maxson et Cie, Paris, 1948.
27. Hildreth, H.M.: Bacteriological Studies of Cl. welchii Infections in Man, Surg. Gyn. Obst., 81: 475-486, 1945.
28. Emard, L.O. and Vaughn, R.H.: Selectivity of Sorbic Acid Media for the Catalase-negative lactic acid bacteria and Clostridia, J. Bact., 63: 487-494, 1952.
29. Humphreys, F.B.: Formation of Acrolein from Glycerol by B. Welchii, J. Inf. Dis., 35: 282-290, 1924.
30. Heller, G., Freeman, M.E., Shope, N.H. and Kindrick, R.H.: Identification of Cl. welchii in Mixed Cultures and Debrided Tissue, and Determination of the Sensitivity of the Organism to Penicillin, Surg. Gyn. and Obstet., 83: 343-347, 1946.

31. Voisenet, E.: Ann. de l'Inst. Pasteur 32: 476, 1918, quoted in Humphreys (29).
32. Instruction Sheet for Brewer Anaerobic Jar. Baltimore Biological Co.
33. Robinson, C.L. and Stovall, W.D.: Stormy Fermentation of Milk in the Recognition of *Cl. welchii* in Wounds, Am. J. Clin. Path., 7:172-183, 1937.
34. Dubos, R.J. and Middlebrook, G.: Cytochemical Reaction of Virulent Tubercle bacilli, Am. Rev. Tuberc., 48: 698-699, 1948.
35. Morse, W.C., Dail, M.C. and Olitzky, I.: A Study of the Neutral-Red Reaction for Determining the Virulence of Mycobacteria, Am. J. Pub. Health, 43: 36-39, 1952.
36. Middlebrook, G., Dubos, R.J. and Pierce, C.: Virulence and Morphological Characteristics of Mammalian Tubercle Bacilli, J. Exp. Med., 36: 175-184, 1947.
37. Wheeler, K.M.: Serological Identification of Dysentery Bacilli, Am. J. Pub. Health, 34: 621-629, 1944.
38. Kauffmann, F.: Zur Serologic der Dysenterie-gruppe, Acta. Path. et microbiol. Scandinav., 19: 53-78, 1942.
39. Edwards, P.R., and Ewing, W.H.: A Manual for Enteric Bacteriology, Communicable Disease Center, Atlanta, Georgia.
40. Fulton, M. and Curtis, S.F.: Bacteriology of a Collection of Shigella Strains Typed by Weil's Method, J. Inf. Dis., 79: 198-203, 1946.
41. Young, V.M.: Geographical Distribution of Shigella, Am. Jour. Trop. Med., 27: 293-299, 1947.
42. Sanders, A.C., Elrod, R.P. and Hullinghorst, R.L.: Shigella Species and Serotypes Identified in Tokyo, Japan, Am. J. Trop. Med., 31: 341-345, 1951.
43. Ewing, W.H. and Gravatti, J.L.: Shigella Types Encountered in the Mediterranean Area, J. Bact., 53: 191-196, 1947.
44. Cox, C.D. and Wallace, G.I.: A Study of Shigella Isolated in India and Burma with Special Reference to Two Previously Undescribed Serotypes, J. Immunol., 60: 465-473, 1947.
45. Weil, A.S.: Dysentery: Progress Report for Years 1942 to 1946, J. Immunol., 55: 363-405, 1947.
46. Boyd, J.S.K.: The Laboratory Diagnosis of Bacillary Dysentery, Tr. Roy. Soc. Trop. Med. Hyg., 33: 553-571, 1940.
47. Mackie, T.T.: The Specificity of the Agglutinin Reaction for *Sh. dysenteriae* II, J. Bact. 37: 27-50, 1939.
48. Ghoda, Akira: Kitasato Institute, Personal Communication, 1952.
49. Boyd, J.S.K.: The Antigenic Structure of the Mannitol-Fermenting Group of Dysentery Bacilli, Jour. Hyg., 38: 477-499, 1938.
50. Weil, A.J., Farsetta, K. and Knaub, V.: Phase Variation of *Sh. paradysenteriae* (Flexner) Type IV, J. Immunol. 52: 221-229, 1946.
51. Wheeler, K.M., Stuart, C.A. and Ewing, W.H.: The Antigenic Complex of the *Sh. paradysenteriae* Boyd Type P. 274, J. Bact., 51: 169-176, 1946.

52. Madsen, S.: On the Classification of the Shigella Types, Ejnar Munksgaarde Forlag, Copenhagen, 1947.
53. Ewing, W.H.: Shigella Nomenclature, J. Bact., 57: 633-638, 1949.
54. Wheeler, K.M.: Antigenic Relationships of Shigella paradysenteriae, J. Immunol., 48: 87-101, 1944.
55. Weil, A.J., de Assis, A. and Slafkovsky, H.: Shigella rio; a new type of Shigella, J. Immunol., 58: 23-26, 1948.
56. Kauffmann, F.: Die Bakteriologie der Salmonella-gruppe, Einar Munksgaard, Kopenhagen, 1-393, 1941.
57. Edwards, P.R. and Bruner, D.W.: Serological Identification of Salmonella cultures, Univ. Kentucky Agr. Exper. Sta. Circ., 54: 1-35, 1942.
58. Lord Stamp and Stone, D.M.: An Agglutinin Common to Certain Strains of Lactose and Non-Lactose-Fermenting Coliform Bacilli, J. Hyg., 43: 266-272, 1944.
59. Frantzen, E.: Biochemical and Serological Studies on Alkalescens and Dispar Strains, Acta. Path. microbiol. Scandinav., 27: 236-248, 1950.
60. Todd, E.W. and Lancefield, R.C.: Variants of Hemolytic Streptococci: Their Relation to Type-Specific Substance, Virulence, and Toxin, J. Exp. Med., 48: 751-767, 1928.
61. Yamamoto, S.: Tokyo University, Personal Communication, 1952.
62. Gauld, R.L., Crouch, W.L., Kaminsky, A.L., Hullinghorst, R.L., Gochenour, W. S. and Yager, R.H.: Leptospiral Meningitis, J.A.M.A., 149: 228-231, 1952.
63. Walch-Sorgdrager, B.: Leptospirosis, League of Nations Bulletin, WHO, 8: 143-386, 1939.

DEPARTMENT OF PATHOLOGY

1. MacLennon, J.D., Anaerobic Infections of War Wounds, Lancet, 2: 63, 1943.
2. Odom, C. D., Causes of Amputation in Battle Casualties, with Emphasis on Vascular Injuries, Surgery, 19: 562, 1946.
3. Power, R. W., Gas Gangrene with Special Reference to Vascularization of Muscles, Brit. M.J., 1: 656, 1945.
4. Neel, H. B. and Cole, J.R. (P), Gas Gangrene in Amphibious Warfare in the Pacific Area, Am. J. Surg., 66: 290, 1944.
5. Langley, F. H. and Winkelstein, L. B., Gas Gangrene, A Study of 96 Cases Treated in an Evacuation Hospital, J.A.M.A., 128: 283, 1945.
6. Hobby, G. L., Meyer, K. and Chaffee, E., Activity of Penicillin in vitro, Proc. Soc. Exptl. Biol. Med., 50: 277-280, 1942.
7. Taylor, W. I., and Novak, M.V., Prophylaxis of Experimental Gas Gangrene in Mice, J. Bact., 61: 571, 1951.
8. Progress Report: Operations at the Renal Insufficiency Center, 1 January to 5 October 1952, Surgical Research Team, 11th Evacuation Hospital, Korea

9. The Physiologic Effects of Wounds by the Board for the Study of the Severely Wounded, North African-Mediterranean Theater of Operation, Office of the Surgeon General, Washington, D.C., 1952.

10. Rather, L. J., Renal Athrocytosis and Intracellular Digestion of Intraperitoneally Injected Hemoglobin in Rats, Jr. Exper. Med., 87: 163, 1948.

11. Faust, E. C., Infection Experiments in Man and Other Mammalian Hosts with Sparganum Stage of Oriental Diphylobothrids, Proc. Soc. Exp. Bio. and Med., 26: 252, 1928.

12. Mueller, J. F., Studies on Sparganum Mansoides and Sparganum Proliferum, A. J. Trop. Med Supplement, 18: 303, 1938.

13. Yokogawa, S. and Kobayashi, H., On the Species of Diphylobothrium mansoni sensu lato, and on the Infectious Mode of Human Sparganosis, Far Eastern Assn. Trop. Med., Trans. 8th Congress, Siam, 1930, 2: 214, 1930.

14. Epidemiology Section, Surgeon's Office, FEC.

DEPARTMENT OF MEDICAL ZOOLOGY

1. McMullen, D.B. A Plate Method of Screening Chemicals as Molluscacides, Jour. Parasit., 35 (Supl.): 28. (Abstract) 1949.

2. Reed, L. J. and Muench, H., A Simple Method of Estimating Fifty Per Cent End Points, Am. J. Hyg., 27 (3): 493-497, 1938.

3. McMullen, D. B., Schistosomiasis and Molluscacides, Am. J. Trop. Med. and Hyg., 1(4): 671-679, 1952.

4. 1951 Annual Historical Report, 406th Medical General Laboratory, 304 pp., 1952.

5. McMullen, D. B., Komiyama, S., Ishii, N., Endo-Itabashi, T., Ozawa, K., Asakawa, T. and Mitoma, Y., The Use of Molluscacides in the Control of Oncomelania nosophora, an Intermediate Host of Schistosoma japonicum, Am. J. Trop. Med., 31 (5): 593-604, 1951.

6. Hunter, G. W. III, Freytag, R. E., Ritchie, L.S., Pan, C., Yokogawa, M. and Potts, D. E., Studies on Schistosomiasis VI Control of the Snail Host of Schistosomiasis in Japan with Sodium Pentachlorophenate (Santobrite). Am. J. Trop. Med. and Hyg., 1(5): 831-847, 1952.

7. Kuntz, R. E., and Wells, W. H., Laboratory and Field Evaluations of Two Dinitro-Phenols as Molluscacides for Control of Schistosome Vectors in Egypt with Emphasis on Importance of Temperature, Am. J. Trop. Med., 31(6): 784-824, 1951.

8. Hoffman, D. O. and Zakhary, R., The Effect of Temperature on the Molluscicidal Activity of Copper Sulfate, Science, 16(Nov.): 521-523, 1951.

9. Sugiura, S., Studies on Biology of Oncomelania nosophora (Robson), an Intermediate Host of Schistosoma japonicum, Mit. Path. Inst. Med. Fakul. Niigata, Japan, 31: 1-18, 1933.

10. Abbott, T., The Egg and Breeding Habits of Oncomelania quadrasi Meldff., the Schistosomiasis Snail of the Philippines. Occ. Papers on Mollusks (Museum of Comp. Zool.), 1(6): 41-48, 1946.

11. McMullen, D. B., The Control of Schistosomiasis japonica I. Observations on the Habits, Ecology and Life Cycle of Oncomelania quadrasi, the Molluscan Intermediate Host of Schistosoma japonicum in the Philippine Islands, Am. J. Hyg., 45(3): 259-273, 1947.
12. Ritchie, L. S., Hunter, G. W. III and Otori, Y., Observations on the Laying and Incubation of Eggs of Oncomelania nosophora, J. Parasit., 37 (Supp.): 16 (Abst.), 1951.
13. Ishii, N. and Tsuda, E., Possibility of the Spreading of Oncomelania nosophora, the Intermediate Snail Host of Schistosoma japonicum, in Other Areas Besides Its Own Habitats, Yokohama Med. Bull., 2(6): 366-375, 1951.
14. Hunter, G. W. III, Freytag, R. E., Ritchie, L.S., Pan, C., Yokogawa, M. and Potts, D. E. (Unpublished Data).
15. Faust, E. C., Human Helminthology, Lea and Febiger, Phila., 3rd Ed., 744 pp., 1949.
16. Iio, S., Studies on the Rapidity of Skin Penetration by Cercariae of S. japonicum; and the Histologic Changes of Host Skin, particularly as Influenced by the Age of the Host (Trans.), Jour. Jap. Soc. of Microbiol. and Path., 22:1299-1303, 1928.
17. Hitchcock, D. J., Penetration Characteristics of Schistosoma mansoni cercariae, J. Parasit., 35(2): 216-217, 1949.
18. Pan, C., Kaufman, E. H. and Hunter, G. W. III, A Technique For Infecting Small Mammals with Schistosoma japonicum, Jour. Lab. and Clin. Med., 37(5):817-819, 1951.
19. Pan, C. and Hunter, G. W. III, A Modified Perfusion Technique for the Recovery of Schistosomes, Jour. Lab. and Clin. Med., 37(5):815-816, 1951.
20. Seckinger, D. L., The Epidemiology of Endamoeba histolytica Infection in Two Rural Georgia Counties, South Med. J. 29(5): 472-477, 1936.
21. Craig, C. F., Etiology, Diagnosis, and Treatment of Amebiasis, Williams and Wilkins, Baltimore, 332 pp, 1944.
22. Mackie, T. T. and Nauss, R. W., Familial Infection by Endamoeba histolytica in New York City, Preliminary Report, Am. J. Trop. Med., 13(6):577-582, 1933.
23. Ritchie, L. S., An Ether Sedimentation Technic for Routine Stool Examinations, Bull. U. S. Army Med. Dept., 8(4): 326, 1948.
24. Faust, E. C., Parasitic Infections in New Orleans, Based on a Cross Section of Charity and Welfare Clinic Patients with Especial Reference to the Children's Clinics, J. Pediat., 2(1): 53-58, 1933.
25. Milam, D. F. and Meleney, H. E., Investigations of Endamoeba histolytica and Other Intestinal Protozoa in Tennessee: II. An Epidemiological Study of Amebiasis in a Rural Community, Am. J. Hyg., 14(2): 325-336, 1931.
26. Meleney, H. E., Bishop, E. L. and Leathers, W. S., Investigations of Endamoeba histolytica and Other Intestinal Protozoa in Tennessee: III. A state-wide Survey of the Intestinal Protozoa of Man, Am. J. Hyg., 16(2): 523-539, 1932.
27. Culbertson, J. T., Immunity Against Animal Parasites, Columbia Press, New York, 274 pp., 1941.
28. Faust, E. C., D'Antoni, J. S., Odom, V., Miller, M. J., Peres, C., Sawitz, W., Thomen, L. F., Tobie, J. E. and Walker, H., A Critical Study of Clinical Laboratory Technics for the Diagnosis of Protozoan Cysts and Helminth Eggs in Feces, Am. J. Trop. Med., 18:169-183, 1938.

29. Tobie, J. E., Reardon, L. V., Bozicevich, J., Shih, Bao-Chih, Mantel, N. and Thomas, E. H., The Efficiency of the Zinc Sulfate Technic in the Detection of Intestinal Protozoa by Successive Stool Examinations, *Am. J. Trop. Med.*, 31(5): 552-560, 1951.

30. Telemann, W., Eine Methode Zur Erleichterung der Auffindung von Parasiteneiern in den Faeces, *Deutsch. Med. Wchnsche*, 34: 1510-1511, 1908.

31. Hunter, G. W. III, Hodges, E. P., Jahnes, W. G., Diamond, L. S. and Ingalls, J. W., Jr., Studies on Schistosomiasis. II. Summary of Further Studies on Methods of Recovering Eggs of *S. japonicum* from Stools, *Bull. U. S. Army Med. Dept.*, 8(2): 128-131, 1948.

32. Matsubayashi, H., Amebiasis and Flagellate Diarrheas, *Igaku-no-shimpo* (Progress of Medicine), No. 5:370-407, 1949.

33. Davis, C. and Ritchie, L. S., Clinical Manifestations and Treatment of Epidemic Amebiasis Occurring in Occupants of the Mantetsu Apartment Building, Tokyo, Japan, *Am. J. Trop. Med.*, 28(6): 817-823, 1948.

34. 1950 Annual Historical Report, 406th Medical General Laboratory, 266 pp., 1951.

FAR EAST MEDICAL RESEARCH UNIT

1. Smadel, J. E., Hertig, M., Traub, R., Steer, A., and Gauld, R. L., Hemorrhagic Fever in a Quartermaster Laundry Company; (A Report to Surgeon, Eighth Army, May, 1952. Study done with Hemorrhagic Fever Research Group and 406th Medical General Laboratory).

2. Gauld, R. L.: Outbreak of Hemorrhagic Fever in an Anti-aircraft Gun Crew; (A Report to Surgeon, Eighth Army, June, 1952; Study done with Hemorrhagic Fever Research Group).

3. Gauld, R. L.: Outbreak of Hemorrhagic Fever in an Infantry Company; (A Report to Surgeon, Eighth Army, June 1952, from Hemorrhagic Fever Research Group).

4. Buescher, E. L.: Epidemiologic Aspects of Epidemic Hemorrhagic Fever; (Unpublished Report, 406th Medical General Laboratory, 1952).

5. Smadel, J. E., Gauld, R. L., Geiman, Q. M., Smithburn, K. C., Hertig, M., Traub, R.: Unpublished Report of Activities of Epidemic Hemorrhagic Fever Field Unit, July 1952.

6. Smorodintsev, A. A., Al'tshuler, I. S., Dunaevskii, M. I., Kokhreidze, K. A., Neustroco, V. D., Churilov, A. V., Etiology and Clinico of Hemorrhagic Nephrosonephritis, *Moscow Medgiz*, 26-27, 1944.

7. Tyzzer, E. E.: Chronic Hepatitis in Infected Animals, *J. Med. Res.*, 37:307, 1917.

8. Oman, Paul W.: Entomologic Investigations of Hemorrhagic Fever (Unpublished Report, 406th Medical General Laboratory, 1952).

9. Traub, R., Hertig, M.: Approach to the Entomological Phases of Epidemic Hemorrhagic Fever; (A Report to Surgeon, Eighth Army, June 1952, from Epidemic Hemorrhagic Fever Field Unit).

10. Traub, R., Hertig, M., Lawrence, W., Harriss, T.: Entomological Observations During Studies on Epidemic Hemorrhagic Fever, 1952; (A Preliminary Report, Field Unit, Epidemic Hemorrhagic Fever Research Group).

11. Earle, D. P.: Disturbed Physiology in Hemorrhagic Fever; A Report to The Surgeon General of Visit to Hemorrhagic Fever Center in Korea, October-December, 1952.
12. Wood, W. Barry: Clinical Aspects of Epidemic Hemorrhagic Fever; A Report to The Surgeon General of Visit to the Hemorrhagic Fever Center in Korea, September and October, 1952.
13. McClure, W. W.: The Use of Nor-Adrenalin in Treatment of Hemorrhagic Fever Patients; (Unpublished Report, 406th Medical General Laboratory, 1952).
14. McDowell, Marion E., Furth, Frank W.: Body Water Compartment Studies in Hemorrhagic Fever. A Report, Army Medical Service Graduate School; (on TDY with Hemorrhagic Fever Center,
15. McDowell, M. E., Froeb, H.: Preliminary Report of Investigation in the Renal Pathophysiology of Hemorrhagic Fever, Army Medical Service Graduate School and 8228th Mobile Army Surgical Hospital; (Investigation at Hemorrhagic Fever Center, 1952).
16. Furth, Frank W.: A Hematologic Survey of Hemorrhagic Fever, Final Report, Army Medical Service Graduate School; (Survey Conducted While on TDY with Hemorrhagic Fever Center, 1952).
17. Langdon, E. A.: p³² Blood Volume Studies in Hemorrhagic Fever; (A Preliminary Report, 1952, Army Medical Service Graduate School, on TDY with Hemorrhagic Fever Center).
18. Steer, Arthur: Pathology of Hemorrhagic Fever; A Comparison of the Findings 1951 and 1952; (Unpublished Report, 406th Medical General Laboratory, 1952).
19. Steer, A., Hullinghorst, R. L.: Epidemic Hemorrhagic Fever, A Special Article. Yearbook of Pathology and Clinical Pathology, The Yearbook Publisher, Chicago: July 1951.
20. Hullinghorst, R. L., Steer, A.: Pathology of Epidemic Hemorrhagic Fever, Ann. Int. Med.: 38: 77-101, January, 1953.
21. Howard, J. M., Scott, R., and Olney, J. M.: Resuscitation of the Severely Injured Battle Casualty; (An Unpublished Report, Surgical Research Team, FEMRU, 1952).
22. The Bacterial Flora of the Battle Wound At the Time of Primary Debridement; (Unpublished Report, 406th Medical General Laboratory and Surgical Research Team, Far East Medical Research Unit).
23. Howard, J. M.: Resuscitation of Battle Casualties; (An Unpublished Report, Surgical Research Team, Far East Medical Research Unit, 1952).
24. Crosby, W. H.: A Study of Blood Transfusion As Used in the Treatment of Battle Casualties in Korea; A Preliminary Report, 1952, from Surgical Research Team and Department of Hematology, Army Medical Service Graduate School).
25. Howard, J. M., Davis J. H., Smith, E. L., and Post, R.: Dehydration Survey in the Combat Soldier; (Report from Surgical Research Team, 1952).
26. Howard, J. M., et al.: Adrenal Function of the Combat Soldier; A Study in the Korean Theater; (Unpublished Report from Surgical Research Team, 406th Medical General Laboratory and Army Medical Service Graduate School, 1952).

27. Jahnke, E. J., and Howard, J. M.: Primary Repair of Arterial Wounds; A Report of Sixty Battle Casualties; (Unpublished Report, Surgical Research Team, Far East Medical Research Unit, 1952).

28. Crosby, W. H., and Akeroyd, J. H.: Some Immunohematological Results of Large Transfusions of Group O Blood in Recipients of Other Blood Groups. A Study of Battle Casualties in Korea, 1952; (Unpublished Report from Surgical Research Team in Korea and Department of Hematology, Army Medical Service Graduate School).

29. Teschan, P. E., et al.: Post-Traumatic Renal Insufficiency; An Outline Summary of Current Concepts. A Dual Report to Surgeon, Eighth Army and Commandant, Army Medical Service Graduate School, 18 March 1953.

OFFICERS AND DEPARTMENT OF ARMY CIVILIANS

1406TH MEDICAL GENERAL LABORATORY AND FAR EAST MEDICAL RESEARCH UNIT

HEADQUARTERS SECTION

Mason, Richard P., Colonel, MC, Commanding
Steer, Arthur, Lt. Colonel, MC, Deputy
Koerner, Charles, Captain, MSC, Executive Officer
Godfrey, William H., Captain, MSC, Adjutant
Hornak, Regina L., 1st Lt., MSC, Personnel Officer
Niiya, Joe, DAC, GS-8
Stein, Glenroy L., DAC, GS-7
Roberts, Dolores M., DAC, GS-6
Lowe, Anne E., DAC, GS-4
Soderstrom, Helen G., DAC, GS-4
Houck, Patricia H., DAC, GS-4

LOGISTICS SECTION

Ruark, Carl D., 1st Lt., MSC, Chief
Jenkins, Arthur D., WOJG
Balazs, Lillian M., DAC, GS-3

BACTERIOLOGY DEPARTMENT

Lindberg, Robert B., Lt. Colonel, MSC, Chief
*Cutchins, Ernest C., Captain, MSC
Newton, Arthur, Captain, MSC
Cardella, Matteo A., 1st Lt., MSC
Jeffries, Charles D., 1st Lt., MSC
*Parrott, Fred C. Jr., 1st Lt., MSC
Thatcher, David W., 2d Lt., MSC
Wetzler, Theodore F., DAC, GS-8
Allen, Josephine, DAC, GS-7
Steinmetz, Jean D., DAC, GS-4

BLOOD BANK

Limbeck, Donald A., Captain, MC, Chief
Rumans, Earl C., Major, MSC
*Griset, Ted A., Captain, MSC

CHEMISTRY DEPARTMENT

Knoblock, Edward C., Major, MSC, Chief
*Carney, Stanis P., Lt. Colonel, MSC
Perella, Dorwin H., Lt. Colonel, VC
Carroll, Nicholas V., Captain, MSC
Cope, Ogle B., Captain, CMLC
Dixon, Arthur C. Jr., Captain, MSC
Pilhorn, Harry R., 1st Lt., MSC
Redfearn, Paul Jr., 1st Lt., MSC
*Haynes, George R., 2d Lt., MSC
Mannering, Gilbert J., DAC, GS-13
Karakawa, James A., DAC, GS-9
Breckinridge, Delora, DAC, GS-4
Shambora, Robert, DAC, GS-1

ENTOMOLOGY DEPARTMENT

Oman, Paul W., Major, MSC, Chief
Graf, Otto N. Jr., 1st Lt., MSC
Scanlon, John E., 1st Lt., MSC
Sicay, Teofilo C., 1st Lt., MSC
Suyemoto, William, DAC, GS-9
Looper, A. Haroldeen, DAC, GS-3
Nishimura, Clara S., DAC, GS-3

EPIDEMIOLOGY DEPARTMENT

McClure, William W., 1st Lt., MC, Chief
Walker, Fawn, DAC, GS-4

MEDICAL ZOOLOGY DEPARTMENT

Ritchie, Lawrence S., DAC, GS-13, Chief
Earle, Hilton H. Jr., Captain, MSC
Wykoff, Dale E., 1st Lt., MSC
Fonseccs, James R.C., 2d Lt., MSC
Williams, James E., DAC, GS-9
Russell, Helen C., DAC, GS-7
Yeager, Frances D., DAC, GS-4

PATHOLOGY DEPARTMENT

Steer, Arthur, Lt. Colonel, MC, Chief
Anderson, Thorwald, Major, MC
Wegner, Calvin J., Major, MC
Vickery, Austin L., Captain, MC
Scully, Robert E., Captain, MC
Handler, Fred P., 1st Lt., MC
LaZerte, Gordon D., 1st Lt., MC
*Rice, William C., 1st Lt., MC
Iwamoto, Meri M., DAC, GS-4
Puglesa, Dolores, DAC, GS-4
Walker, Rosemary, DAC, GS-4

SEROLOGY DEPARTMENT

Stein, George J., DAC, GS-14, Chief
Gelsing, Harry P., DAC, GS-9
Bigley, Mildred, DAC, GS-4

VIRUS AND RICKETTSIAL DEPARTMENT

Buescher, Edward L., Captain, MC, Chief
Byrne, Robert J., Captain, VC
Cousins, Richard F., Captain, MSC
Goldfein, Samuel, Captain, MC
*Philpot, Van Buren, Captain, MC
*McCollum, Robert N., 1st Lt., MC
Rosenberg, Murray Z., 1st Lt., MC
*Kain, Herbert K., 2d Lt., MSC
McClure, H. Elliott, DAC, GS-12
Paul, Gloria, DAC, GS-6
Dimont, Bertha, DAC, GS-4
Nakai, Hisayo, DAC, GS-4

* Assigned for training

RESEARCH AND DEVELOPMENT TEAMS FROM OSG AND AMSGS:

EPIDEMIC HEMORRHAGIC FEVER FIELD UNIT

Dr. Joseph E. Smadel, Chief, Division of Communicable Diseases, Army Medical Service Graduate School
Dr. Marshall Hertig, Medical Entomologist, Ancon, Canal Zone, Panama
Dr. Ross L. Gauld, Chief, Department of Epidemiology, Army Medical Service Graduate School
Dr. Quentin M. Geiman, Department of Tropical Public Health, Harvard School of Public Health
Dr. Kenneth C. Smithburn, Division of Medicine and Public Health, The Rockefeller Foundation
Dr. David P. Earle, Jr., Associate Professor of Medicine, New York University College of Medicine
Dr. W. Barry Wood, Professor of Medicine, George Washington University
Dr. Arnold V. Wolf, Physiologist, Army Medical Service Graduate School
Dr. Joel E. Warren, Bacteriologist, Army Medical Service Graduate School
Dr. Louis J. Lipovsky, Entomologist, University of Kansas
Dr. Douglas J. Gould, Entomologist, Army Medical Service Graduate School
Miss Elizabeth B. Jackson, Bacteriologist, Army Medical Service Graduate School
Lt. Colonel Robert Traub, MSC, Army Medical Service Graduate School
Major Edward A. Langdon, MC, Army Medical Service Graduate School
Major Marion E. McDowell, MC, Army Medical Service Graduate School
Captain Thomas T. Harriss, MSC, Brooke Army Medical Center
Captain William H. Lawrence, MSC, United States Army Hospital, Camp Stewart, Georgia
1st Lieutenant Frank W. Furth, MC, Army Medical Service Graduate School

SURGICAL RESEARCH TEAM

Captain John M. Howard, Director, Surgical Research Team, Army Medical Service Graduate School
Lt. Colonel William H. Crosby, MC, Army Medical Service Graduate School
Major Edward J. Jahnke, USAF, Army Medical Service Graduate School
Captain John H. Davis, MC, Brooke Army Medical Center
Captain Roy L. Mundy, MSC, Army Medical Service Graduate School
Captain Lloyd H. Smith, Jr., MC, Army Medical Service Graduate School
Captain Paul E. Teschan, MC, Army Medical Service Graduate School
1st Lieutenant Michael Ladd, MC, Army Medical Service Graduate School
1st Lieutenant Russel Scott Jr., MC, Army Medical Service Graduate School
1st Lieutenant Donald L. Leanard, MSC, Army Medical Service Graduate School
1st Lieutenant John M. Olney, MC, Army Medical Service Graduate School
1st Lieutenant Robert S. Post, MC, Army Medical Service Graduate School
1st Lieutenant Joseph C. Strawitz, MC, 406th Medical General Laboratory

WOUND BALLISTICS AND BODY ARMOR TEAM

Lt. Colonel Robert H. Holmes, MC, Armed Forces Institute of Pathology
Lt. Colonel William W. Cox, MC, Office of the Surgeon General
Major William F. Enos, MC, Armed Forces Institute of Pathology
Captain James C. Beyer, Armed Forces Institute of Pathology
Captain Joseph V. Michalsky, Army Chemical Center, Maryland
1st Lieutenant George B. Coe, MSC, Army Chemical Center, Maryland
1st Lieutenant Richard B. Stoughton, MC, Army Chemical Center, Maryland

NEUROPSYCHIATRIC RESEARCH UNIT

Dr. David McK. Rioch, Neuropsychiatrist, Army Medical Service
Graduate School

Captain Roger W. Little, MSC, Department of Sociology and Anthropology, Michigan State College

CONSULTANTS:

Colonel William S. Stone, MC, Commandant, Army Medical Service
Graduate School

Dr. Francis D. Moore, Surgeon-in-Chief, Peter Bent Brigham
Hospital, Boston

Dr. Jesse E. Edwards, Mayo Clinic, Rochester, Minnesota, Consultant
on Pathology to the Surgeon General

Dr. Anthony Curreri, Consultant in Surgery to the Surgeon General

Dr. Robert D. Dripps, Consultant in Anaesthesiology to the Surgeon
General

Dr. Everett W. Jameson, Jr., Division of Zoology, University of
Southern California

Dr. Chapman H. Binford, Medical Director, United States Public
Health Service

Colonel Hugh R. Gilmore, Jr., MC, Chief of Pathology and Allied
Sciences Division, Office of the Surgeon General

Colonel William H. Amspacher, Chief, Surgical Research Team,
Brooke Army Medical Center

